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Systematics of the *Viola nuttallii* Complex

by

© D. M. Fabijan

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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Date December 12, 1983

Abstract

Morphological, cytological and phytochemical data were incorporated into numerical and statistical analyses resulting in a revision of *Viola* subsection Nuttallianae. Five species, with three subspecies, were recognized. A basic chromosome number of $x = 6$ was confirmed in this polyploid complex which includes: *V. vallicola*, $2N = 12$; *V. tomentosa*, $2N = 12$; *V. nuttallii*, $2N = 24$; *V. praemorsa*, $2N = 36$ and 48; and, *V. bakeri*, $2N = 48$. A total of twenty-nine flavonoid compounds were found in leaf tissue extracts from these eight taxa. Identified flavones and flavonols included the arabinose, galactose, glucose, rhamnose, and rutin glycosides of apigenin, kaempferol, luteolin and quercetin. The number of different flavonoid compounds per taxon increased with ploidy level within this complex.

V. vallicola subspecies *vallicola* possessed primarily apigenin glycosides and occurred in Alberta and Saskatchewan. Subspecies *major*, on the other hand, was found to possess predominently kaempferol glycosides and occurred only in the interior of British Columbia. A number of specimens of*V. vallicola* from the Great Basin regions of Idaho and Nevada displayed intermediate flavonoid profiles. The derivation of the subspecies is hypothesized to be a result of isolation on either side of the continental divide during post-glacial northward migration.

V. nuttallii possessed apigenin, kaempferol and quercetin flavonoids suggesting that it is an allotetraploid of *V. vallicola* subspecies *vallicola* and and *V. tomentosa* which also possessed apigenin, kaempferol and quercetin derivatives. *V. tomentosa* has since become restricted in range to the Sierra Nevada mountains and *V. nuttallii* is found only on the east side of the Rocky Mountains. *V. vallicola* has the widest distribution of any of the Nuttallianae, ranging from southern British Columbia to Manitoba and south to Arkansas and Nevada.

V. bakeri and *V. praemorsa* possessed the most diverse flavonoid profiles, up to twenty per taxon, including luteolin aglycone and glycosidic derivatives. Presence or absence of different flavonoid aglycones appeared to be random in the specimens in these taxa. The three subspecies of *V. praemorsa*: *praemorsa*, *linguaefolia*, and *flavovirens*,were recognized by their morphological characteristics, though they appear to represent extremes in a continuous range of variation. The existence of hybrids

supports the hypothesis that other previously recognized subspecific taxa ; subspecies *arida* and *oregona*, are the result of hybrid crosses of *V. praemorsa* and *V. bakeri* or with members of closely related subsections.

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I. INTRODUCTION

A. General Introduction

The *Viola nuttallii* complex of the family Violaceae has been identified as a polyploid series; the taxa in this group contain meiotic chromosome complements of 6, 12, 18, and 24. Due to their morphological similarities, they have been difficult to distinguish, resulting in the description of twenty different taxa since *Viola nuttallii* was described in 1814. The *Viola nuttallii* complex thus provided a challenging taxonomic problem.

1. The Family Violaceae

The family Violaceae consists of 16 genera and approximately 800 species of worldwide distribution (Cronquist, 1981). Fourteen of the sixteen genera in the family are restricted to the southern hemisphere and particularly to South America.

Melchior (1925) divided the family into two subfamilies based primarily on floral characters. Leonideae have terminally opening stamens and nut-like fruit, while the Violoideae have introse stamens and capsular fruit. The subfamily Violoideae was further divided into two tribes; the Violeae Gingins was distinquished from the Rinoreae Reiche and Taubert by the possession of zygomorphic flowers in which the lower petal was saccate or spurred. The tribe Violeae was further divided into the subtribes Hybanthinae with two genera, and the Violinae with five genera including *Viola* L. The Violinae are distinguished by a relatively long spurred anterior petal and distinct stamens with appendages extending into the spur.

2. The Genus *Viola* L.

The nearly 400 species of the family Violaceae belong to the genus *Viola*, which is composed primarily of annual and perennial herbs, although shrubby and arboresent species are found in South America (Camp, 1947). Whereas actinomorphous flowers predominate in other genera of the Violaceae, those of the genus *Viola* L. are strongly irregular (Clausen, 1929; Cronquist, 1981).

Camp (1947) proposed Central and South America to be the area of origin of the Violaceae and of the genus *Viola*. He based this hypothesis on the southern distributions of many of the genera in the family, particularly the *Rinorea* which were considered primitive within the family (Cronquist, 1981).

The only genus within the family which is best developed in north temperate regions and in tropical mountains is *Viola* (Figure 1). Its South American members have been shown to possess the greatest number of primitive character states that are most closely related to other members of the Violaceae (Clausen, 1929). Camp (1947) contended that *Viola* was "evolutionarly preconditioned" at high elevations in the Andes for its dispersal via Mexico and North America, into the northern hemisphere.

The approximately 400 species in the genus *Viola* were divided into 14 sections by Becker (1925). Six sections occur in the northern hemisphere, and of these, three are found in North America (Valentine, 1962). The sections Chamaemelanium Ging. (Figure 1) and Viola are distributed in both Asia and North America, while Melanium Ging. is primarily European with one species occuring in North America.

3. The section Chamaemelanium

The section Chamaemelanium contains the greatest number of western North American *Viola* species (Baker, 1949c). Violets of the Chamaemelanium section are distinguished from those of other northern sections by their small stipules, yellow or yellowish flowers with capitate styles, and the presence of cleistogamous flowers on aerial stems (Becker, 1925; Baker, 1935 and 1949c).

Two classifications of the subsections in the Chamaemelanium are shown in Table 1.

Clausen (1964) recognized three Chaemaemelanium subsections: the Pedunculatae, the Purpureae, and the Nuttallianae whereas Becker (1925) had grouped all of the species into one subsection, Nuttallianae. The Nuttallianae and Purpureae, which were both composed of large species complexes, had previously been grouped into one subsection, Nuttallianae (Baker, 1949c), which was otherwise identical to Clausen (1964).

The Nuttallianae are distinguished by the presence of glabrous capsules as compared to the appressed, puberulent capsules of the Purpureae; by generally lacking

Figure 1. The worldwide distribution of *Viola* and the section Chamaemelanium Ging. (Clausen, 1929; Camp, 1947). (adapted from)

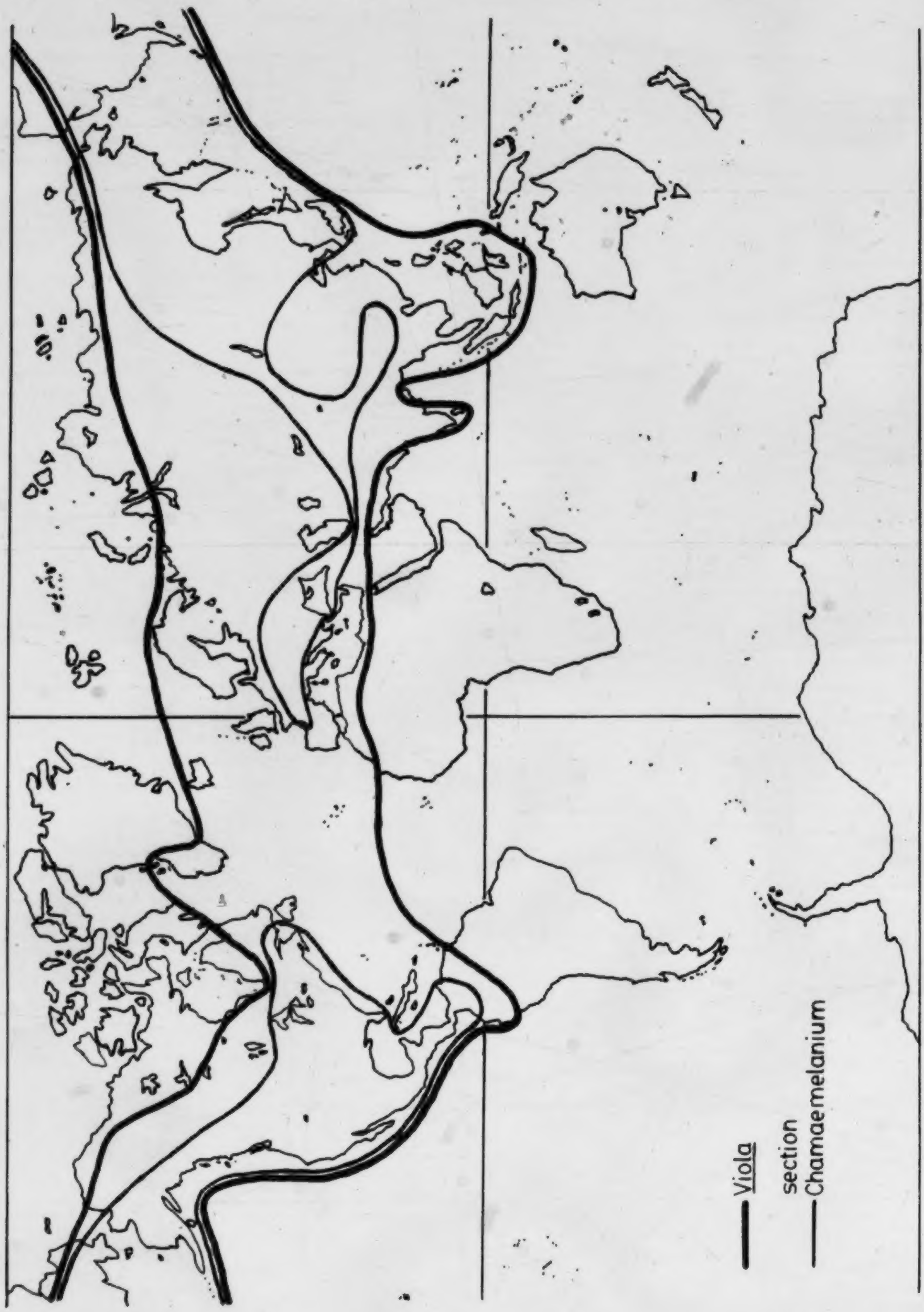
Viola
section
Chamaemelanium

Table 1. The subsections of the Chamaemelanium Ging. (Becker, 1925; Clausen, 1964).

Becker	Clausen
Barroetanae Bckr.	
Nuttallianae Bckr.	Nuttallianae (Bckr.) Cl. Purpureae Cl. Pedunculatae Cl.
Chrysanthae Bckr.	Chrysanthae Bckr.
Erectae - Monophyllos Bckr.	
- Nudicaulis Bckr.	Nudicaulis Bckr.
- Canadensis Bckr.	Canadensis Bckr.
Flagelliformes Bckr.	
Section Dischidium Ging.	Biflorae Cl.

elongated aerial stems; and, by size and shape differences in the basal leaves (Baker, 1957; Clausen, 1951; 1964). Further, in contrast to the Purpureae (n = 6), the Nuttallianae constituted a polyploid species complex with chromosome numbers of n = 6, 12, 18 and 24.

Members of the Chamaemelanium section found in western North America and Mexico may represent the prototypes of the temperate *Viola*, constituting a link between South American tropical members and the other northern temperate violets (Clausen, 1929; 1964). The species of the Chamaemelanium were judged to be the most primitive of the *Viola* on the basis of their low chromosome number of n = 6, and the presence of variations in style shape, stigma form, and leaf form found in other sections (Clausen, 1929). Cytological and morphological data coupled with geographic distribution led to a phylogenetic proposal for the northern violets by Clausen (1929), in which the Chamaemelanium section was thought to be the central group from amounst whose members the ancestors of the other northern subsections and sections might have been found.

B. The *Viola nuttallii* Pursh Complex

1. Historical Introduction

Viola nuttallii Pursh was first collected by Thomas Nuttall in 1811 while he was on the Astor Expedition on the Missouri River. Pursh's (1814) publication described a pubescent unbranched plant having ovate-oblong, nearly entire leaves with long gradually tapering petioles.

In 1829, Lindley published the description of a new species by D. Douglas, *Viola praemorsa* Dougl. ex Lindl. It was named for its truncate rootstock, and was distinguished from *V. nuttallii* by possessing denticulate, ovate-oblong leaves covered by hirsute hairs. *V. praemorsa* was found to be abundant on the dry plains of the Columbia River and especially along the Pacific Coast. It was only one year later that Hooker (1930) included in this species a rare form from the Columbia River area, *V. nuttallii* Pursh variety *major* Hook. with wide leaves and large flowers and growing in the shade of pines. He stated that this taxon was related to the collections of Nuttall from the

Missouri River. Hooker also synonymized *V. glareosa* Dougl. with variety *major*, though it was never published.

The Flora of North America by Torrey and Gray (1843) included yet another species description by Nuttall of a plant which he collected in 1834 while accompanying the Wyeth Expedition. *V. linguaefolia* Nutt. ex T. & G. possessed pubescent, oblong-lanciolate and somewhat serrate leaves, being intermediate between those of *V. nuttallii* and *V. praemorsa*.

Based on collections of Heller in Idaho in 1896, Pollard (1897) described *V. flavovirens* Poll. as a tall yellowish-green plant covered with fine hirsute pubescence.

In 1898, *V. bakeri* Greene was described as a pubescent form with entire, ciliate margined leaves intermediate between *V. nuttallii* and *V. praemorsa* from California.

V. vallicola A. Nels. was not described until Nelson (1899) distinguished it from *V. praemorsa* by its finely puberulent or glabrous character and peduncles which did not elevate the flowers above the height of the leaf blades. In 1900 Nelson described another related species from subalpine habitats in Wyoming and Idaho. This species, *V. erectifolia* A. Nels., was distiguished from *V. nuttallii* by its erect leaves being somewhat wider and with a single vertical taproot. Otherwise, *V. erectifolia* appeared to be very similar to the *V. nuttallii* represented by the drawing in Hooker (1830).

Other species continued to be described as related to *V. nuttallii*, for example: *V. gomphopetala* Greene and *V. physalodes* Greene (Greene, 1901), *V. subsagittifolia* Suks. and *V. xylorrhiza* Suks. (Suksdorf, 1927), and *V. russellii* Boivin (Boivin, 1951). New combinations also began to appear for previously described taxa: *V. nuttallii praemorsa* (Piper, 1906), *V. nuttallii linguaefolia* Piper (Piper and Beattie, 1914), *V. nuttallii* var. *vallicola* (St. John, 1937), *V. praemorsa* var. *linguaefolia* (Peck, 1941), to mention but a few.

2. Cytological Investigations

With the advent of cytological methods, new emphasis was placed on chromosome numbers in taxonomic work. Amongst the first plants investigated were members of the genus *Viola* (Miyaji, 1913, in German translation, 1930). Miyaji, in a study of Japanese violets, found meiotic chromosome numbers of 6, 12, 18, 24 and 36 present in the genus. The cytology of North American violets was first investigated in conjunction with the hybridization studies occuring in the early years of this century (Brainerd, 1904; Gershoy, 1928; 1934).

It was also at this time that Milo Baker, in collaboration with Jens Clausen, began a revision of western North American violets using cytological techniques. Their determinations, which were included in numerous publications between 1935 and 1960, were subsequently summerized with documentation by Clausen (1964). As a result, a number of new combinations were made and new taxa described. *V. tomentosa* Baker (1949a), n = 6, a rare endemic of the Sierra Nevada was similar to *V. bakeri*, n = 24, and *V. nuttallii*, n = 12, but with densely tomentose leaves and capsules. *V. bakeri* subspecies *grandis* Baker (1949a), was described as a larger leaved form of *V. bakeri*. *V. praemorsa* subsp. *linguaefolia* (Nutt.) Baker and Clausen (Baker, 1957), n = 18, and *V. praemorsa* subsp. *major* (Hook.) Baker and Clausen (Baker, 1957), n = 24, represent two new combinations. *V. praemorsa* subsp. *arida* Baker (1957), n = 24, was very similar to subsp. *praemorsa* except for its short and sparse pubescence on leaves and capsules. *V. praemorsa* subsp. *oregona* Baker (1957), n = 24, was found in a few isolated areas in southern Oregon and northern California; it differed from *arida* by having spreading instead of erect foliage, and minutely puberulent capsules.

To this point each taxon was represented by only one or very few chromosome counts. Davidse (1976), in the most recent work on this group, undertook an extensive cytological investigation of the members of the Chamaemelanium section, including some Nuttallianae, in the Intermountain Region between the coastal Sierra Nevada mountains and the Rocky mountains. He confirmed all of the above reports except for *linguaefolia* for which he acquired nine counts of n = 24 (Table 2).

3. Classification of the Nuttallianae

As a basis for discussion, and by way of a summary of the most extensive work done to date, the classification of Clausen (1964) will be reviewed. This classification included *V. tomentosa* Baker, *V. bakeri* Greene, *V. nuttallii* Pursh, *V. vallicola* A. Nels., *V. linguaefolia* Nutt. ex T. & G., and *V. praemorsa* Dougl. ex Lindl. with three subspecies: *praemorsa*, *major* (Hook.) Baker and Clausen, and *oregona* Baker and Clausen.

Table 2. Cytology of the Nuttallianae and reported hybrids.

Taxa	N	Reference
Viola tomentosa	6	Baker, 1949a and b
V. bakeri	24	Baker, 1949b
V. vallicola	6	Baker, 1949b; Davidse, 1976
V. nuttallii	12	Baker, 1949b
V. linguaefolia	18 24	Baker, 1949b Gershoy, 1934; Davidse, 1976
V. praemorsa		
subspecies *praemorsa*	15 18	Gershoy, 1928 Gershoy, 1934; Baker, 1949b
subspecies *major*	24	Baker, 1949b; Davidse, 1976
subspecies *oregona*	24	Baker, 1949b
Reported Natural Hybrids		
V. praemorsa		
subspecies *major X V. utahensis* (Purpureae)	18	Davidse, 1976
subspecies *major X V. douglasii* (Chrysanthae)	24	Clausen, 1964
V. tomentosa X V. purpurea (Purpureae)		
subspecies *purpurea*	-	Baker, 1949a; Clausen, 1964
subspecies *integrifolia*	-	Baker, 1949a; Clausen, 1964
V. tomentosa X V. sheltonii (Chrysanthae)	-	Baker, 1949a; Clausen, 1964

The classification of the Nuttallianae by Clausen (1964) has not, however, been accepted universally. Several authors have chosen to designate all taxa uniformly as varieties (Hitchcock and Cronquist, 1961; Scoggan, 1978) or subspecies (Peck, 1961) of *V. nuttallii* due to the lack of sufficient cytological data and the absence of reliable morphological or geographical differences in the cases of some taxa.

In his synopsis of the western North American violets, Clausen (1964) concluded that the 33 taxa within the Chamaemelanium section belonged to at least seven polyploid lines which represented distinct evolutionary sequences. The subsection Nuttallianae comprised two of these polyploid lines, based on two geographically separated diploid species.

The first polyploid line was based on *V. tomentosa*, n = 6, a rare endemic of the Sierra Nevada (Baker, 1949a). This line was thought to be more closely related to the subsection Purpureae (*V. purpurea*, *V. quercetorum*, and *V. utahensis*) than to other members of the Nuttallianae. *V. tomentosa* was presumed most closely related to *V. bakeri*, n = 24, based on morphological characters, although no hexaploid species was known with which it could have hybridized to produce *V. bakeri*.

The second series included *V. vallcola*, *V. nuttallii*, *V. praemorsa*, and *V. linguaefolia*. Clausen suggested that this line arose from *V. vallicola*.

V. nuttallii, n = 12, is a prairie species that remains east of the continental divide in a range extending from the Canadian prairie provinces as far south as Arizona and as far east as Nebraska. It is distinguished by its lanceolate leaves which are attenuated to a long petiole and minutely puberulent throughout, and by arising from a deep-seated rootstock with renewal buds below the soil surface.

V. vallicola, n = 6, has the widest distribution of any taxon in the complex. It is sympatric with *V. nuttallii*, but also ranges into central British Columbia, Washington and Oregon. It has a truncated, vertical rootstock and ovate of oblong leaves which tend to have truncate or subcordate bases (Baker, 1957).

Baker (1957) suggested that *V. nuttallii* was an autotetraploid of *V. vallicola*, although Clausen (1964) questioned this conclusion based on their morphological distinctiveness. He suggested that an undiscovered diploid *V. nuttallii* might exist from which the known tetraploid arose via autopolyploidy. No such diploid has been discovered and its existence was considered to be unlikely by Davidse (1976).

V. praemorsa subspecies *praemorsa*, n = 18, occurs in the humid regions of the Pacific coast from California to southwestern British Columbia. It was named for the praemorse habit of its roots and is distinguished by its dense, hirsute pubescence throughout, its oblong-ovate leaves with truncate bases, and flowers on peduncles which surpass the leaves in height.

V. linguaefolia is a montane to subalpine violet of the Great Basin Ranges and Rocky mountains, extending northward into Alberta, geogrphically separated from subspecies *praemorsa*. It possesses ovate leaves with cuneate bases that are between *V. vallicola* and *V. nuttallii* in form. This suggested to Clausen (1964) that *linguaefolia* may be a natural amphiploid of these two species.

V. praemorsa subspecies *major*, n = 24, was morphologically very difficult to distinguish from *V. linguaefolia*, n = 18, the only reliable means being cytological. Clausen found it to differ from subspecies *praemorsa* by its erect ovate or oblong leaves which equal or surpass the flowers in height. Subspecies *major* is found from Washington to central California through the Cascade and Sierra Nevada mountains.

Baker (1949b) reported *V. linguaefolia* to be a hexaploid, n = 18, like *V. praemorsa* subspecies *praemorsa*. He postulated that *praemorsa* probably differentiated from *linguaefolia* as a result of westward dispersal through the Columbia Gap to the coast (Baker, 1957). Clausen (1964), on the other hand, suggested that the rise of the Cascade mountains lead to the present geographic separation of these two hexaploids which were once connected across the low plains.

Davidse (1976) could not corrobarate early reports of n = 18 for *linguaefolia* from nine populations in Utah, Nevada, and Idaho. Each population had a count of n = 24 with regular meiosis and high pollen fertility. As no reliable morphological or geographical differences could be found, he considered *V. linguaefolia* to be synonymous with *V. praemorsa* subspecies *major*. Davidse further suggested that the previous count of n = 18 from Utah may have been based on a hybrid between *major* and *V. utahensis* Baker and Clausen (subsection Purpureae), similar to the one reported by him (Table 2).

V. praemorsa subspecies *oregona* has a chromosome complement of n = 24 and reportedly occurs in limited areas of southern Oregon and northern California. Leaves of this taxon are grayish in colour due to a continuous coat of pubescence and similar in form to the ovate- or oblong-lanceolate leaves of *V. bakeri*. *Baker (1957) referred to oregona* as a subspecies in the making and that as such, it was probably the youngest taxon of the *V. praemorsa*.

C. Objectives

The major objective of this study was to attempt to construct a revised classification of the *Viola nuttallii* complex. Related to this was the need to investigate the relationship between *V. vallicola* and *V. nuttallii*, and, between these taxa and the remainder of the group.

In order to achieve this, a cytological survey of the members of this complex was undertaken, particularly of those members which occur in Canada. Detailed morphological investigations of the characteristics of this group were also carried out in order to establish a usable classification based on readily observable features which are statistically significant in distinguishing between taxa. Flavonoids present in the leaf tissue of the members of the *V. nuttallii* complex were also examined in order to supplement and corroborate the morphological data.

Numerical techniques provide a powerful tool by which to analyze complex phenetic relationships among groups of organisms (Sokal and Sneath, 1978). Although the resultant classification would be based on data not ordinarily used in routine field identification, it must be correlated with readily observable morphological characters to be of general use. Numerical techniques have the ability not only to supply a classification but also to statistically determine which characters are "good" in the taxonomic sense. These objectives were based on the need to use a holistic approach, that is, to investigate as many aspects of the problem as was practical.

The operational species concept applied in this study was a practical one, following that of Cronquist (1978): "species are the smallest groups that are consistently and persistently distinct, and distinguishable by ordinary means." This concept incorporates the working assumptions of many plant taxonomists. The smallest groups are those which cannot be further subdivided and still conform to the definition. Consistent features exhibited by an individual must fit into the range of variation within the group, and, group variation must have a discontinuity with the variation exhibited by other groups. These group variations must be reasonably persistent between generations and must be distinguishable by means ordinarily used by a botanist, that is, using a hand lens. Reproductive isolation is implied by the definition, since phenetic discontinuity cannot be maintained in the absence of some barrier to interbreeding.

For the remainder of this discussion, the taxa in question will be refered to by their specific epithets only, to avoid any confusion as to their taxonomic status.

II. Materials and Methods

A. Herbarium Loans

Specimens from the following herbaria were examined: Arnold Arboretum of Harvard University (A); University of Alberta (ALTA); British Museum, London, England (BM); National Museums of Canada (CAN); Dudley Herbarium (DS) and California Academy of Sciences (CAS), Stanford University; Agriculture Canada (DAO); Gray Herbarium of Harvard University (G); University of Idaho (ID); Jepson Herbarium, University of California (JEPS); KEW Botanical Gardens, England (KEW); Missouri Botanical Garden (MO); University of Pennsylvania (PENN); Academy of Natural Sciences of Philadelphia (PH); Rocky Mountain Herbarium, University of Wyoming (RM); W.P. Fraser Herbarium, University of Saskatchewan (SASK); University of Calgary (UAC); University of British Columbia (UBC); University of California (UC); Smithsonian Institute, U.S. National Herbarium (US); University of Regina (USAS); University of Victoria (UVIC); British Columbia Provincial Museum (V); Willamette Herbarium (WILLU) of Oregon State University (OSU); and, Marion Ownbey Herbarium, Washington State University (WS).

Abbreviations follow those used by Holmgren and Keuben (1974).

B. Collections

1. Field Studies

Field collections were made throughout as much of the reported range of the *V. nuttallii* complex as was possible during the spring and summer of 1981 and 1982. This included the Canadian range, extending from Vancouver Island through southern British Columbia, Alberta, and Saskatchewan; and selected sites in the United States Pacific Northwest including Washington, Oregon, California, Idaho, and Montana. At each collection site, representative members of the population were sampled and pressed for voucher specimens. Others were collected in bulk and air dried for chemical analysis. Pollen and flower buds were sampled and preserved. At least two live plants were potted for transplantation. All materials were subsequently transported to the University of Alberta for cultivation in the greenhouses, deposition of voucher specimens, and/or detailed chemical and morphological analysis.

2. Greenhouse Cultivation of Live Material

Plants in the greenhouse were maintained under a 16 hour photoperiod throughout the year, supplemented with high intensity discard (HID) lamps which provided a minimum intensity of 80 $uE.m^{-2}s^{-1}$. Temperature was maintained at 18° C during the day and 15° C during the night. Relative humidity was held constant at 60%.

Some plants were overwintered naturally outside while others were induced into dormancy and stored in a freezer (McPherson, 1972). The dormancy induction schedule covered nine days during which the plants were brought from 18° to -2° C: three days at 12°, two days at 8°, three days at 2 to 4° in darkness, then watered well, placed in sealed plastic bags and stored at -1° to -4° in the dark. After five weeks, the plants were removed from the freezer and returned directly to the greenhouse growing regime. The survival of plants subjected to both natural and artificial overwintering conditions was about 35% for *V. vallicola* and *V. nuttallii*. *V. praemorsa* specimens, which grew well under greenhouse conditions, died back to the roots and regenerated repeatedly throughout the year, and were not subjected to overwintering procedures. *V. nuttallii* and *V. vallicola* also underwent a period of dormancy naturally in the greenhouse but did not regenerate until the following March.

The overall survival of collected specimens following transplantation and cultivation was about 80%. Difficulty of cultivation of these taxa over long periods was reported by Baker (Clausen, 1964).

Seeds were collected from cultivated plants by bagging the expanding capsules in muslin, allowing for retention of the seeds after the dehiscence of the capsule.

3. Cytological Studies

Mitotic chromosome counts were made on root tip squashes prepared from actively growing roots using a modification of the method of Tijo and Levan (1950). Root tips were treated in a 0.002M solution a 8-hydroxyquinoline for 2 to 2.5 hours at 14 to 17° C. Tips were washed in distilled water for 10 minutes, transfered to a watch glass containing aceto-orcein and 1N hydrochloric acid (9:1 by volume) for 30 minutes.

The solution was warmed over an alcohol flame briefly 3 to 5 times in 30 minutes. Tips were then placed on a microscope slide in 45% acetic acid. All but the apical 1mm of the root tip was removed, a cover slip applied, and the preparation was squashed by tapping the coverslip several times. Semi-permanent slides were made by ringing the coverslip with a melted mixture of gum mastic and parafin wax (2:1 by volume). Chromosomes were examined under oil emersion with a green filter using an Olympus BHA,PM-10-M photomicrographic system and photographed with an Olympus C-35 camera.

Cytological examination of pollen mother cells was carried out using the iron-aceto-carmine method (Clausen, 1929; Radford et al., 1974; Sharman and Sharma, 1980). Flower buds were collected in the field and fixed in Carnoy's solution (chloroform:ethanol:acetic acid, 4:3:1 by volume) then transferred to 70% ethanol and stored at -4° C until slide preparation and examination.

4. Pollen Viability Tests

Pollen was collected in the field by dissecting anthers from chasmogamous flowers and placing them directly into small vials containing lacto-phenol cotton blue stain (Radford et al, 1974). Vials were stored under refrigeration for up to four months prior to examination. Pollen stored in this manner showed no significant difference in viability or size from that stained and examined fresh. Pollen was examined at 400X magnification; the diameter was measured using a calibrated occular micrometer.

Viability was estimated as the percentage of pollen grains that were stained dark blue in the 100 grains examined. The diameter of 100 viable pollen grains was measured in up to five pollen samples collected at random from each flowering population. Significance of means was tested using the t-test (Campbell, 1974).

5. Morphological Measurements

Characters were selected on the basis of previous authors' treatments of the *nuttallii* complex (Baker, 1935, 1949b; Clausen, 1964); those used in other descriptions of the taxa; and, through the examination of a wide range of living and herbarium material. As a result, 19 morphological characters were selected for scoring the specimens. Each quantitative character was measured a minimum of ten times. Characters were scored at the same relative position and developemental stage on each plant.

Measurements of leaf characters (length, width, and basal angle), flower size (length of lower petal including the spur in chasmogamous flowers), length of cleistogamous sepals, length of leaf pubescence, and presence or absence of pubescence on sepals and capsules were made from voucher specimens. Seeds produced by cleistogamous and chasmogamous flowers showed no significant differences in morphological characteristics (length, width, weight, colour, and caruncle size); therefore, no distinction was made between them. Data represent means of from 2 to 100 seeds, the total being dependent on the number of seeds produced by each plant cultivated in the greenhouse facilities.

6. Scanning Electron Microscopy

A Cambridge Spectroscan 250 was used to make detailed examinations of pubescence and surface characteristics of both seeds and pollen. Samples were glued to the specimen stud using two-sided adhesive tape. The material was treated for four minutes in plasma mode in a sputter coater, shadowing it with approximately 15 nm of gold prior to examination.

C. Flavonoid Chemistry

1. Paper Chromatography

A survey of flavonoids from all field collections and selected herbarium specimens was carried out by extracting 25 mg of dry, powdered leaf tissue in 2 ml of 80% methanol overnight on a shaker. One ml of this extract was spotted dropwise on 46 by 57 cm Whatman 3MM chromatography paper. Each chromatograph was subjected to two dimensional descending liquid chromatography. The first solvent was butanol:acetic acid:water, BAW (4:1:5 by volume, upper phase), and the second was 15% acetic acid. Each chromatogram was dried and rotated 90° between the two solvent runs. The resulting chromatograms were examined for colour under ultra-violet light (3660 A); in the presence of ammonia vapor and UV light; and, after spraying with NA reagent (diphenylboric acid-2-amino ethyl ester in methanol).

Tissue from five representative populations (D.F. collections 478, 507, 532, 537, and 648) was extracted for identification of constituent flavonoids. Five grams of dried, powdered leaf tissue from each sample were extracted with shaking in 100 ml of 50% methanol overnight, filtered *in vacuo* and re-extracted in the same manner in 80% methanol. The two filtrates from each sample were bulked, and the aqueous phase reduced to a minimum, at 55° C *in vacuo* using a Buchi rotary evaporator.

At least 36 replicates of each extract were spotted and chromatographed in BAW and Acetic acid as previously described. For each extract, only those compounds from the resultant chromatograms which showed a positive colour reaction to NA reagent during the preliminary survey were investigated further.

For each sample, each compound was coded, the spots on the chromatographic paper cut out, and extracted overnight with shaking in 100% methanol. The extracts were filtered *in vacuo* and evaporated to near dryness at 55° *in vacuo* on a rotary evaporator.

Each compound was checked for purity by subjecting it to one-dimensional descending paper chromatography on 23 by 57 cm Whatman 1MM paper in each of four solvent systems: BAW (A); distilled water (B); 15% acetic acid (C); and, 80% phenol in water (by volume)(D) as described in Harborne (1967). Rf values for each compound in each of these four solvent systems were then determined in comparison to Rutin which was run concurrently on each chromatograph. Any further necessary purification was carried out on Whatman 1MM paper using the solvent system which gave the best separation.

Thin layer chromatography (TLC) was also employed on the purified extracts using four different systems. Polyamid-6 (MN Polygram, 0.1 mm thick) plastic TLC plates were run in two separate systems. System E was an aqueous solvent consisting of water: ethanol:methylethyl ketone (70:20:10 by volume) which took about 90 minutes to run 10 cm. The organic solvent (System F), 1,2-dichloroethane: methylethyl ketone:water (50:25:21:4 by volume) took about 65 minutes to run 10 cm. Cellulose plates (DC-fertigplatten PEI-Cellulose) were run in 15% acetic acid for 10 cm, taking about 50 minutes (System G). Silica gel G plates on glass (System H) were run in a solvent system of methylethyl ketone:acetic acid:water (85:12:3, by volume) for 10 cm which took about 45 minutes.

Thus, each extracted and purified compound was run in eight different paper and thin layer chromatographic systems.

2. Spectral Analysis

Flavonoids exhibit characteristic absorbance peaks when examined in UV and visible light. The two major absorption peaks occur in the 300-400 nm (Band I) and 240-280 nm (Band II) range and are associated with substitutions on the B- and A- rings, respectively, of the flavonoid structure. A flavonoid compound may be characterized by first determining its absorption spectrum in absolute methanol, and secondly, by the addition of five diagnostic reagents: powdered sodium methoxide (NaOMe), to saturation; 6 drops of methanol saturated with anhydrous aluminum chloride ($AlCl_3$); 3 drops of 30% hydrochloric acid (HCl); powdered sodium acetate (NaOAc); and, boric acid (H_3BO_3) as described by Mabry et al., (1970). These spectra were obtained from paper chromatographically purified compounds on a Unicam SP1800 spectraphotometer. Compounds were identified by comparison to published spectral and chromatographic data (Mabry et al., 1970; Jay et al., 1975)

3. Hydrolysis of Flavonoid Glycosides

Hydrolysis of the sugar component of the flavonoid glycosides was carried out under acidic conditions. A portion of the purified extract was transfered to a test tube in methanol. A maximum of 5 ml of 10% hydrochloric acid was added, the test tubes were covered with aluminum foil, and heated at 100° C in a water bath for 90 minutes. On cooling, this mixture was partitioned three times with 2 mls of amyl alcohol, and both fractions were then evaporated to dryness. The alcohol fractions , which contained the aglycones, were redissolved in absolute methanol and subjected to the same spectral analysis described previously.

The acidic aqueous fractions containing the sugar residues were then chromatographed, with standards, on silica gel (DC Fertiplatten S.1 G-25) thin layer chromatography plates. These were chromatographed twice in the same solvent system: ethyl-acetate:isopropanol:water (65:22:11 by volume); then, air dried, sprayed with aniline phthalate reagent (aniline, 4.6 g; Phthalic acid, 8.0 g; n-butanol, 245 ml; ether, 245 ml; and, water, 10 ml), and developed by heating at 100° C for 15 to 30 minutes.

Identification of sugars was accomplished by comparison of Rf values to known standards which were chromatographed at the same time. The most common flavonoid sugar residues, glucose, galactose, rhamnose, xylose and arabinose, were used as standards.

D. Numerical Analysis

Fifty chemical and morphological characters were employed in the statistical and numerical analyses (Table 3) including 48 continuous and two classed characters.

Flavonoid characters were scored as quantitative characters on a continuous scale of 0 to 3 where 0 was absent, and 3 indicated a relatively dark spot was present on the given chromatographic paper. All flavonoids reacting positively to NA reagent were included whether or not they were successfully identified.

Chromosome number and seed colour were included as unordered classed characters though it could be argued that they were continuous. By recording these attributes as unordered classes one avoided the problems of *a priori* designations of linear relationships between the character states and of unequal spacing or unequal size of the classes (Sneath and Sokal, 1973).

The Operational Taxonomic Unit (OTU) was defined here as the sum of all individuals observed from an isolated population of plants, including the voucher specimen, living material, and dried material used in chemical analysis. The major criteria for including OTUs in the numerical analysis was the availability of cytological data, though this requirement was waived in the case of type specimens.

The numerical analysis was performed at two levels: on the individual OTUs to investigate the validity of the taxa, and on the taxa for evaluating phenetic and chemical interrelationships. The individual OTU analysis included cytologically known specimens for which flavonoid analysis was available and selected type specimens for which only limited morphological data could be acquired.

The classification program used to aid in establishing taxa was TAXMAP, a program developed by Dr. J. W. Carmichael of the University of Alberta. TAXMAP was employed in preference to the other available classification programs for a number of

Table 3. Characters used for numerical analysis.

No.	Type[1]	Character name and states	95% CL
1	2	K[2] 3-O rut (0,1,2,3: absent to very dark)	2.00
2	2	A 7-O rut	2.00
3	2	A -	2.00
4	2	K 6-Me,3-O rut	2.00
5	2	-	2.00
6	2	Q 3-O rut	2.00
7	2	Q 3,7-O digly	2.00
8	2	Q 3-O monoglu	2.00
9	2	K 3,7-O rhagal	2.00
10	2	-	2.00
11	2	L 7-O monogal	2.00
12	2	-	2.00
13	2	L aglycone	2.00
14	2	A 7-O monoglu	2.00
15	2	L 7-O monoglu	2.00
16	2	A 6-Me, 7-O ara,glu	2.00
17	2	L 7-O triglu	2.00
18	2	-	2.00
19	2	-	2.00
20	2	-	2.00
21	2	-	2.00
22	2	-	2.00
23	2	-	2.00
24	2	-	2.00
25	2	-	2.00
26	2	-	2.00
27	2	Q 3-O digly	2.00
28	2	-	2.00
29	2	-	2.00
30	5	2N Chromosome number (1,2,3,4: 12,24,36,48)	0.10
31	2	Cauline leaf length (mm)	4.00
32	2	Cauline leaf width (mm)	2.00
33	2	Cauline leaf length / width ratio	0.42
34	2	Cauline leaf basal angle (°)	16.00
35	2	Pubescence length (mm)	0.05
36	2	Margin serration (0,1,2,3: entire to serrate)	0.80
37	2	Margin ciliation (0,1: absent, present)	0.80
38	5	Sepal pubescence (0,1,2,3: absent, auricles, ciliate, all)	0.80
39	2	Petal length (mm)	0.86
40	2	Cleistogamous sepal length (mm)	0.42
41	2	Capsule pubescence (0,1: absent, present)	0.80
42	2	Seed length (mm)	0.09
43	2	Seed width (mm)	0.70
44	2	Seed length / width ratio	0.12
45	2	Seed weight (mg)	0.28
46	2	Caruncle length (mm)	0.10
47	2	Caruncle length / seed length ratio	0.05
48	2	Caruncle covering seed apex(0,1: absent, present)	0.10
49	6	Seed colour (1,2,3,4,5,6: tan, light brown, brown, dark brown, red brown, white)	0.10
50	2	Pollen diameter (um)	0.72

[1] Weighting: 2 = log 2 of number of confidence interval classes in range. Includes continuous state and ordered classes
3 to N - unordered classes; match weight = log 2 N, no-match weight = 1.0

[2] Compounds: A = Apigenin, K = Kaempferol, L = Luteolin, Q = Quercetin, gly = glycoside, glu = glucose, rha = rhamnose, gal = galactose, rut = rutin, - = unidentified.

reasons related to the nature of the data used in this study. TAXMAP is versatile in its requirements for data types, it can handle continuous or real number data, ordered classes and unordered classes simultaneously. Distance calculations can incorporate inherent information content provided by the confidence intervals. A very important feature is the ability to handle missing data.

TAXMAP calculates the ranges of the attribute values, normalizes the raw data as fractions of the range, and then calculates a relative distance between each pair of OTUs. TAXMAP also allows for distance calculations to be made by the weighting of attributes according to their relative information content as given by 95% confidence limits. Both equally weighted and weighted analyses were used. The clustering procedure as outlined by Carmichael (1980), was illustrated by means of a taxometric map (Carmichael and Sneath, 1969). Taxometric maps were drawn with the aid of the Calcomp plotter at the University of Alberta Computing Services or by hand based on the information provided by TAXMAP. The map represents a two-dimensional display of the multi-dimensional hyperspace in which the clusters exist.

Three TAXMAP analyses were performed. Firstly, weighted and equally weighted analyses based on all 50 attributes were made of the 59 OTUs representing the author's collections. It was felt that this represented the most reliable analysis based on the greatest available information. A second analysis making use of morphological data only (21 attributes) was compared to the first analysis. The third analysis was run to include a number of herbarium specimens for which cytological data were available and flavonoid analysis was possible, and a number of type specimens for which only limited morphological data were attainable. This brought the total number of OTUs to 95 (Table 4).

Table 4. Specimens used in TAXMAP analysis.

OTU Number	OTU Name[1]	Location	2N
1	1-572	Chin Lake, 23km S Taber, Alberta. 3000′.	12
2	4-524	Nose Hill, Calgary, Alberta. 3700′.	12
3	9-550	Little Fish Lake, Alberta. 3600′.	12
4	15-567	Spruce Coulee, Cypress Hills, Alberta. 3600′.	12
5	34-516	Leitch Collieries, Crowsnest Pass, Alberta. 4500′.	12
6	35-517	1km S of Bow Crow Forest Entrance, S of Hillcrest, Alberta. 5500′.	12
7	36-519	Lynx Creek, 19km S of Hillcrest, Alberta. 4500′.	12
8	37-520	Castle River, S of Hillcrest, Alberta. 4500′.	12
9	38-523	Beauvais Lake, Alberta. 4500′.	12
10	54d-533	Nose Hill, Calgary, Alberta. 3700′.	12
11	56-551	Hand Hills, N of Little Fish Lake, Alberta. 3000′.	12
12	57-579	1.8km SW of Cypress Hills Park, Saskatchewan. 4100′.	12
13	58-590	3.7km S of Moose Jaw, Saskatchewan.	12
15	10-556	3.0km E of Cereal, Alberta. 2600′.	12
16	25-545	0.5km W of Turner Valley, Alberta. 4000′.	12
17	22-489	3.6km E of Cawston, British Columbia. 3000′.	12
18	23-487	Courtney Lake, B.C. 3500′.	12
19	24-488	Princeton, B.C. 2500′.	12
20	50-471	57.7km S of Washington state border, Oregon hwy#3.	
21	52-486	11.4km S of Kamloops toward Lac La Jeune, B.C.	12
22	53-507	13.3km E of Grand Forks, B.C. 2000′.	12
23	21-499	Johnston Creek Park, B.C. 3000′.	12
24	6a-570	N bank of Bow River, Scandia, Alberta. 2400′.	24
25	8-538	Cochrane Hill, Cochrane, Alberta. 4000′.	24
26	13-571	N bank of Old Man River, Taber, Alberta. 2500′.	24
27	16a-573	Etzikom Coulee, 38.6km S of Taber, Alberta. 3100′.	24
28	17-576	Writting-on-Stone, Alberta. 3000′.	24
29	54a-537	Nose Hill, Calgary, Alberta. 3700′.	24
30	59-596	3.5km S and 2.3km W of Wood Mountain Park, Saskatchewan.	24
31	26-253	Fabyan, Alberta. 2100′.	24
32	27-531	18km N of Wainwright, Alberta. 2000′.	24
33	62-622	Red Rock Pass, Idaho. 7000′. **Type site** - *erectifolia*	48
34	63-631	McDonald Pass, Montana. 6325′.	48
35	64-648	Carthew Summit, Waterton, Alberta. 7500′.	48
36	65-654	Boivin Lake, Alberta. 6800′.	48
37	51-478	1.5km S and 9.6km E of Anatone, Washington.	36
38	40-375	Klickitat Valley, N of Maryhill, Washington.	48
39	41-394	2.7km N and 4.3km W of Lyle, Washington.	36
40	42-396	Trout Lake, Washington. 12,325′.	48
41	43-398	5.8km E of Husum, Washington. **Type site** - *xylorrhiza*	36
42	44-414	Tacoma, Washington. **Lectotype site** - *typica*	36
43	45-442	Greensprings Mountain, E of Ashland, Oregon. 4550′.	36
44	46-443	Klamath River, E of Klamath Falls, Oregon. **Type site** - *oregona*	36
45	47-458	Klamath Falls, Oregon. **Type site** - *arida*	48
46	48-459	Kneeland, California..	36
47	49b-463	Howard's Gulch, 4km NW of hwy#229 on hwy#139, California.	36

48	5b-548	5.7km E of Cluny, Alberta. 2800'.	12
49	7-568	Whitla Coulee, 0.3km E of Etzikom turnoff on hwy#3, Alberta. 2700'.	12
50	12-565	4.6km N of Golden Prairie turnoff on hwy#41, Alberta. 2700'.	12
51	14-566	Medicine Hat, Alberta. 2300'.	12
52	30-547	Bow Crow Forest entrance, 10km W of Turner Valley, Alberta.4800'.	12
53	31-276	Waterton, Alberta. 4400'.	12
54	29-268	8.0km W of Longview, Alberta. 4300'.	
55	16b-194	Etzikom Coulee, 38.6km S of Taber, Alberta. 3100'.	12
56	28-256	Porcupine Hills, 8.7km E of hwy#22 towards Nanton, Alberta. 4500'.	12
57	5a-549	5.7km E of Cluny, Alberta. 2800'.	24
58	55-546	Turner Valley, Alberta. 4100'.	24
59	2-112	Expanse Coulee above Old Man River, Alberta. 2500'.	
60	B9634	(MO)[2] Tacoma, Washington. April, 1940. **Lectotype** - *praemorsa* Baker	36
61	B8059	(CAS) Humbolt County., California. April, 1935.	36
62	D1738	(MO) Elko Co., Nevada. May, 1969.	48
63	B8385	(DS) Sierra Co., California. June, 1936	48
64	B8403	(DS) Placer Co., California. June, 1936.	48
65	B7354	(CAS, MO) Siskiyou Co., California. April, 1933.	48
66	B8388	(DS) Sierra Co., California. 6770'. June, 1936.	48
67	B5215	(DS) Placer Co., California. May, 1936.	48
68	B8406	(DS) Nevada Co., California. 6000'. June, 1936.	48
69	B8377	(DS) Tehama Co., California. 6000'. June, 1936.	48
70	D1746	(CAS, MO) Owyhee Co., Idaho. May, 1969.	48
71	GD1640	(CAS, MO) Humbolt Co., Nevada. 6960'. June, 1967.	48
72	B8052	(DS) Mount Lassen, California. 5800'. June, 1935.	48
73	B11462	(DS, MO) Klamath River, Oregon. May, 1946. **Isotype** - *oregona* B. & Cl.	48
74	B12086	(DS, CAS) Klamath Falls, Oregon. April, 1949. **Isotype** - *arida* B. & Cl.	48
75	B7408	(CAS, MO) Kamiah, Idaho. 1500'. April, 1933.	48
76	A8317	(DS) Klamath Co., Oregon.	48
77	B9359	(CAS, MO) Ruby Mountains, Nevada. June, 1939.	12
78	D1754	(MO) Blaine Co., Idaho. May, 1969.	12
79	D1732	(MO) Elko Co., Nevada. May, 1969.	12
80	D1753	(MO) Camas Co., Idaho. May, 1969.	12
81	D1736	(MO) Elko Co., Nevada. May, 1969.	12
82	D1745	(MO) Owyhee Co., Idaho. May, 1969.	12
83	B8699	(DS) Sierra Co., California. 5400'. July, 1937.	12
84	HOOK/K	(KEW)Camas Prairie near source of Columbia. 1834. **Type** - *major* Hook.	
85	AN4340	(PH) Pole Creek, Wyoming. May, 1894. **Co-type** - *vallicola* Nels.	
86	S10200	(PH) Husum, Washington. June, 1920. **Type** - *xylorrhiza* Suks.	
87	S8530	(PH) Spangle, Washington. May, 1916. **Type** - *subsagittifolia* Suks.	
88	CFB67	(DS) Amarron, Colorado. 6900'. 1901. **Type** - *physalodes* Greene	
89	RS-BS	(DAO) Duval, Saskatchewan. May, 1942. **Type** - *russellii* Boivin	
90	ANSIPH	(PH) Missouri. **Type** - *nuttallii* Pursh	
91	B8662	(MO) 20.5 mi. W of Klamath Falls, Oregon. May, 1937. **Type** - *oregona* B. & Cl.	48

92	AN5481	(WS) Continental Divide near Henry's Lake, Idaho. **Type** - *erectifolia* Nels.
93	CFB225	(WS, RM) Grand Mesa, Colorado. 9000'. June, 1902. **Type** - *gomphopetala* Greene
94	H3156	(WS, DAO, MO, US) Lake Waha, Nez Perce Co., Idaho. 3500-4000'. June, 1896. **Type** - *flavovirens* Poll.
95	SMH222	(US, UC, GH) Craig Mountains, Nez Perce Co., Idaho. 900m. May, 1892. **Co-type** - *flavovirens* Poll.

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[1] Unless otherwise specified, all collection numbers are the author's and consist of a site number-collection number designation. A = E. Applegate, B = M. S. Baker, D = G. Davidse, GD = J. Gentry and G. Davidse, S = W. Suksdorf, AN = A. Nelson, RS-BB = R. S. Russell and B. S. Sallons, HOOK = W. Hooker, CFB = C. F. Baker, H = Heller.

[2] Herbaria from which leaf tissue was obtained for chemical extraction, or where type specimens are located.

III. Results

A. Cytology and Palynology

In his 1964 review, Clausen expressed the need for an extensive cytological investigation of the Chamaemelanium in order to provide a more firm basis for the classification of this section. In this study 59 new chromosome counts; were made 57 for the Nuttallianae subsection, one in the Purpureae subsection, and one possible hybrid (Table 5). Voucher specimens are deposited at the University of Alberta (ALTA).

Somatic chromosome numbers of 2N = 12, 24, 36, and 48 were found. That these counts are all multiples of six supports the hypothesis that the base chromosome number for the Nuttallianae subsection is $x = 6$. When compared to reports of other chromosome numbers in the Chamaemelanium the base number for the entire section is likely to be 6 (Davidse, 1976; Clausen, 1964), contrary to the earlier work of Clausen (1927, 1929), which was based on very few counts.

Measurements of pollen size and estimates of pollen viability were made on cytologically known specimens (Figure 2). A t-test indicated no significant difference (P = 0.01%) in pollen diameter or viability estimates for pollen from cleistogamous versus chasmogamous flowers on the same plant (data not shown). All measurements reported here were subsequently made using only pollen from chasmogamous flowers.

The results from stored and fresh pollen measurements were compared for selected samples using the paired sample t-test (Campbell, 1974). At the P = 0.1% level, no significant difference was evident in either size or viability, though pollen grains stored in stain tended to be slightly larger than those examined within a few hours of staining (data not shown).

For within-population viability, 2 to 7 measurements from selected populations were examined (data not shown). Standard deviation, SD, for any given measurement was about 9% and the standard error, SE, 2%. Standard deviation, however, could range from 0 to 41% within a population. Within a given sample, pollen size varied about 2.5 um SD (0.5 um SE) and within-population variation was only slightly higher (data not shown).

Table 5. New chromosome counts in the *Viola nuttallii* complex and other Chamaemelanium. All collections were made by the author.

2N	Location	Site Number	Collection Numbers
Subsection Nuttallianae			
Viola vallicola			
12	ALBERTA		
	West bank of Chin Lake	1	105,572
	Nose Hill, Calgary	4	126,524
	Cluny	5b	134,548
	Whitla Coulee	7	148,568
	Little Fish Lake	9	150,550
	Cereal	10	155,556
	Golden Prairie	12	165,565
	Medicine Hat	14	178,566
	Spruce Coulee, Cypress Hills	15	181,567
	Etzikom Coulee	16	194,574
	Turner Valley	25	233,545
	Porcupine Hills	28	256,244
	Bow Crow Forest	30	270,547
	Crownest Pass, Leitch Collieries	34	295,516
	Hillcrest	35	296,517
	Lynx Creek Campground	36	297,519
	Castle River near bridge	37	300,520
	Beavais Lake Park	38	311,523
	Nose Hill, Calgary	54d	532
	Hand Hills NE of Little Fish Lake	56	551
12	BRITISH COLUMBIA		
	Kettle River	20	224
	Johnston Creek Park	21	499
	Cawston Road to Oliver	33	229,489
	Courtney Lake	23	230,487
	West of Princeton	24	232,488
	Lac La Jeune	52	486
	E of Grand Forks	53	507
12	SASKATCHEWAN		
	W of Cypress Hills	57	579
	S of Wood Mountain	58	590
	S of Moose Jaw	60	604
Viola nuttallii			
24	ALBERTA		
	Cluny	5a	128,549
	Scandia	6	145,570
	Cochrane Hill, Cochrane	8	149,538
	Empress	11	161,561
	Taber	13	170,571
	Etzikom Coulee	17	193,573
	Writting-on-Stone Park	17	198,576
	Fabyan	26	253,530
	Wainwright	27	531
	Nose Hill, Calgary	54a	537
	Turner Valley	55	546

24	SASKATCHEWAN		
	Wood Mountain	59	596

Viola praemorsa

36	WASHINGTON		
	Lyle	41	394
	Husum	43	397,398,399
	Tacoma	44	414
	Anatone	51	478
36	OREGON		
	Green Springs Mountain	45	442
	Klamath River	46	443,444
36	CALIFORNIA		
	Kneeland	48	459
	Howard's Gulch	49B	463
48	WASHINGTON		
	Klickitat Valley north of Maryshill	40	375
48	OREGON		
	Klamath Falls	47	458
48	IDAHO		
	Red Rock Pass	62	622
48	MONTANA		
	McDonald Pass	63	631
48	ALBERTA		
	Carthew Summit, Waterton	64	648
	Bovin Lake	65	654

Viola Bakeri

48	WASHINGTON		
	Trout Lake	42	395

Subsection Purpureae

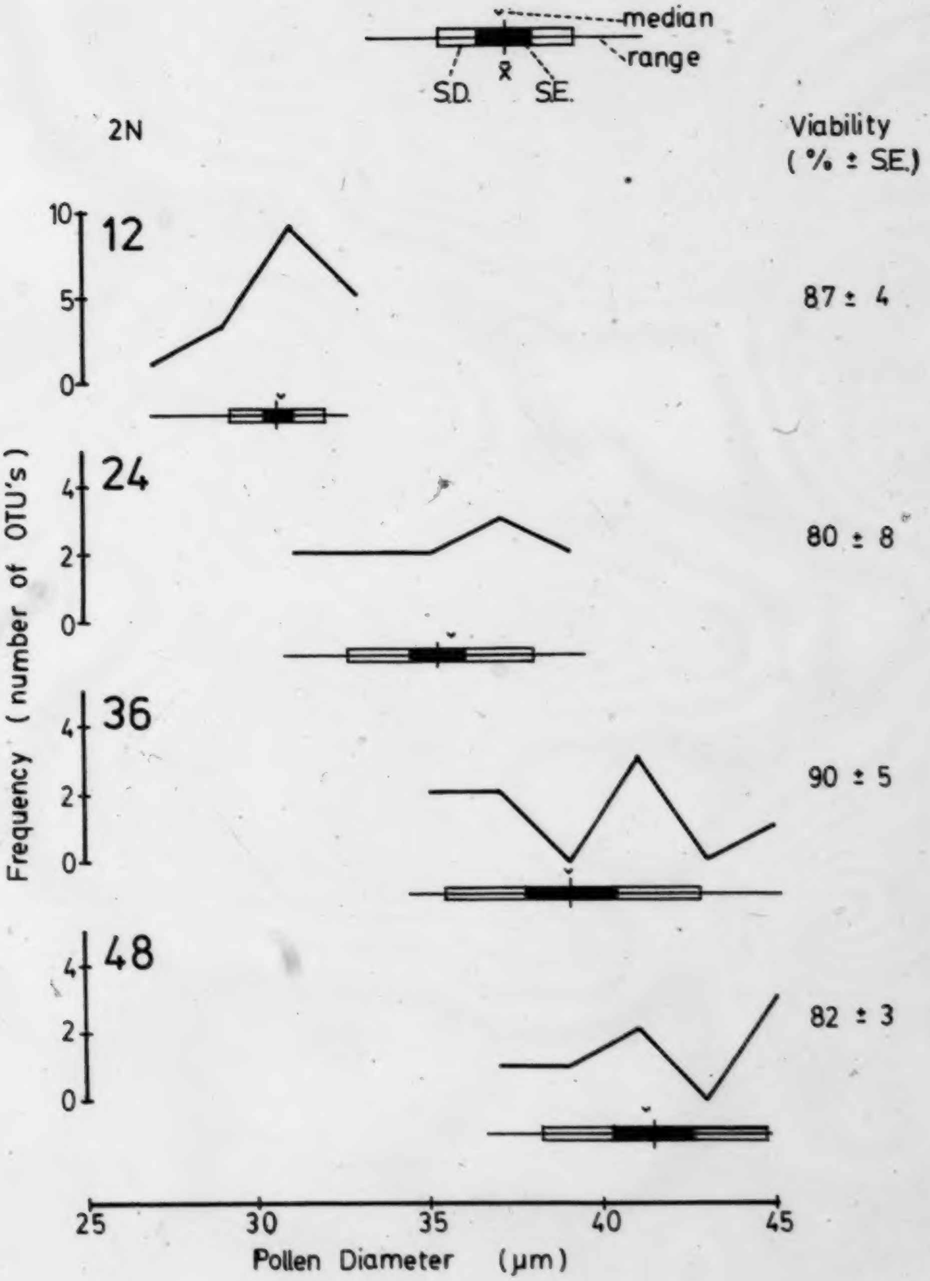
Viola purpurea

12	CALIFORNIA		
	Howard's Gulch	49A	462

V. purpurea X V. praemorsa

28	CALIFORNIA		
	Howard's Gulch	49C	462a

Figure 2. Pollen grain diameter and viability estimates for the chromosome races in the *Viola nuttallii* complex.



Pollen viability estimates were generally over 80% for each chromosome class (Figure 2). Very low viability measurements obtained in some cases were likely due to adverse environmental conditions in the field. Where viability estimates were low for pollen samples collected in the field, pollen from the same plant under cultivated conditions typically showed viability greater than 80%. Investigators, therefore, should be wary of basing conclusions regarding hybridization solely on low pollen viability without testing interpopulation measurements and insuring that these measurements are made under "ideal" conditions.

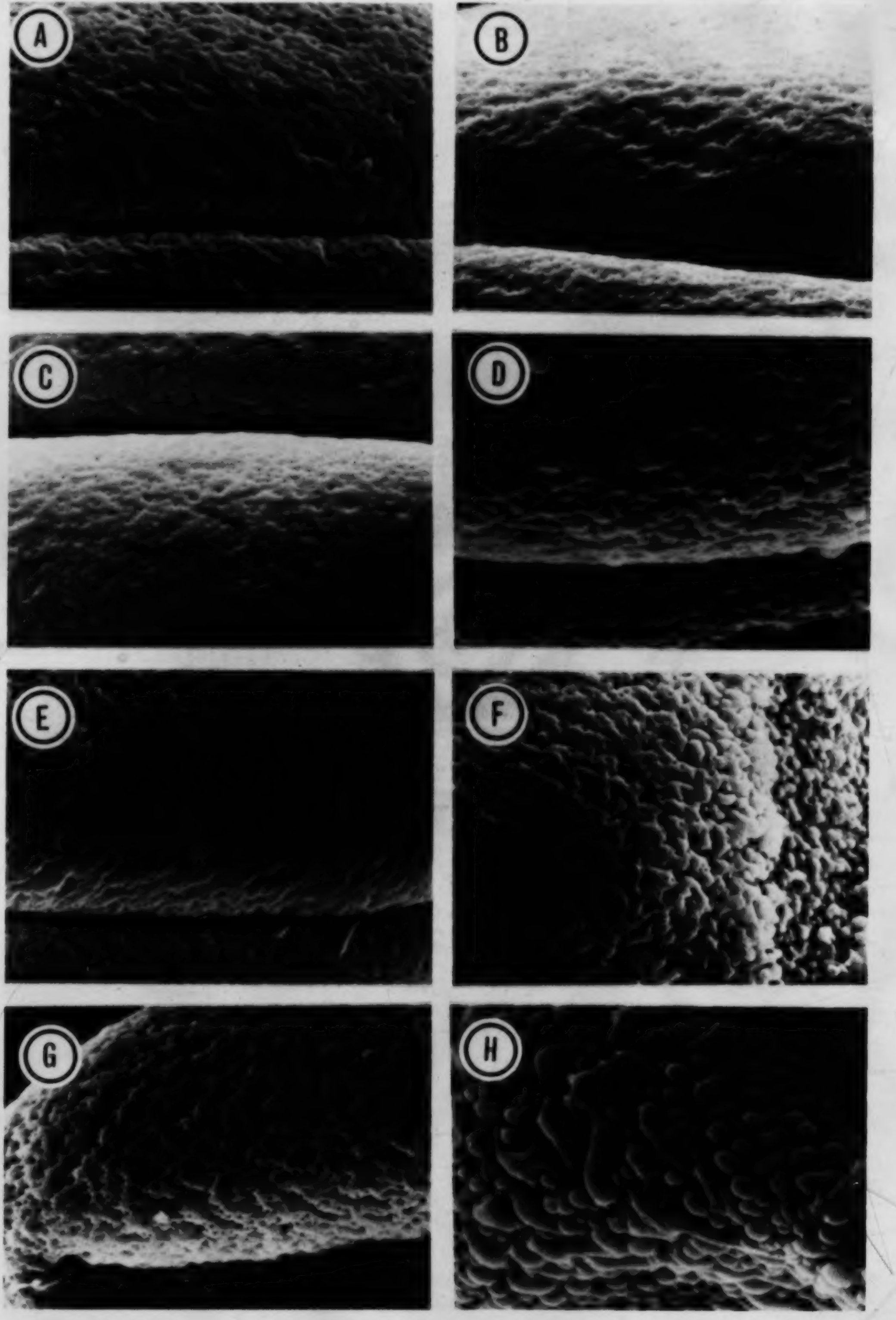
Pollen diameter increased with ploidy level (Figure 2). Pollen size for the two ploidy levels 2N = 12 and 24 were found to be significantly different (P = 1%) from each other and from the other ploidy levels using the Kruskal-Wallis analysis of variance of ranks (Campbell, 1976). No significant difference (P = 1%) was found between the size of pollen grains in the 2N = 36 and 48 ploidy levels. Their ranges overlapped significantly. When comparing the frequency graphs, each may be composed of two or three size classes. Conversely, a given pollen size class may represent two ploidy levels.

Scanning Electron Microscopy (SEM) of selected pollen samples confirmed light microscope observations that these medium sized grains were tricolpate with granulate aperture membranes (Figure 3). In the light microscope observations, pollen in polar view appeared to be triangular to circular in shape. SEM observations of untreated pollen grains from voucher specimens revealed elliptic pollen grains as seen in equatorial view, indicating that dry pollen shrinks to a fusiform appearance. The foveolate or pitted surfaces vary in the density and size of pits and in the presence of scabration or rugulation.

Pollen from cleistogamous and chasmogamous flowers of 15 specimens were examined. In some specimens, some minor differences in pollen grain surface sculpturing were observed between cleistogamous versus chasmogamous flowers (data not shown). No consistant variation was detectable in this limited study due to the small sample size, though there may be a trend toward an increase in the size of scabrae and rugulation with increased chromosome number.

Figure 3. Scanning Electron Microscopy of *Viola nuttallii* complex pollen grains. Specimens for each photo are indicated by the site number and collection number; all collections were made by the author.

A. *V. vallicola*, 2N = 12. (DF 50-471)
B. *V. vallicola*, 2N = 12. (DF 52-486)
C. *V. nuttallii*, 2N = 24. (DF 8-538)
D. *V. bakeri*, 2N = 48. (DF 42-396)
E. *V. praemorsa* supspecies *praemorsa*, 2N = 36. (DF 44-414)
F. *V. flavovirens*, 2N = 36. (DF 51-478)
G. *V. praemorsa* subspecies *arida*, 2N = 48. (DF 47-458)
H. *V. linguaefolia*, 2N = 48. (DF 64-648)


A
B
C
D
E
F
G
H

In summary, pollen grain size provided a reliable means of distinguishing two of the ploidy levels and was therefore incorporated into the numerical analysis. Details of pollen surface features were not included in the subsequent numerical analysis. More extensive sampling would be required in order to do this.

B. Morphology

1. Floral Characteristics

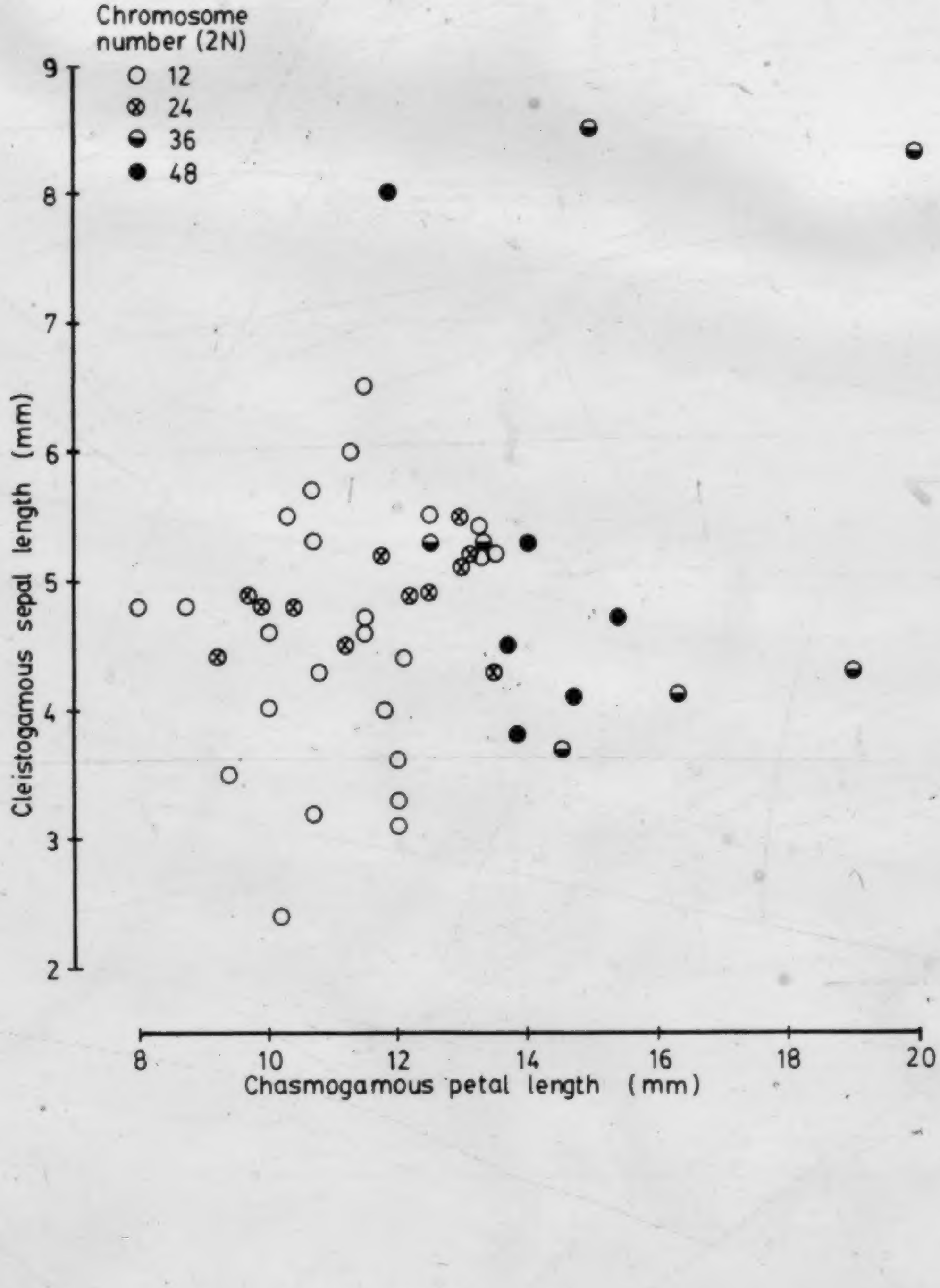
Measurements of flower size were carried out on well pressed herbarium specimens and compared to those of fresh flowers. Significant shrinkage occured in even the best pressed specimens; thus only measurements of live material were included here. Using the total length of the lower, spurred petal as an indicator of chasmogamous flower size (Figure 4), it was evident that, although there was some overlap, there were two distinct classes of petal size. The chasmogamous petal length of the 2N = 12 and 24 chromosome groups was about 11.5 mm, while those of the 36 and 48 chromosome levels ranged much larger, from 13.5 to 20 mm long. Thus, this attribute may be useful in distinguishing these two chromosome groups.

No such reliable separation was evident in the measurement of cleistogamous flower size as measured by the length of sepals (Figure 4). Though little variation occured within the 12 and 24 chromosome groups, the sepal length means were not significantly different. A great deal of variation occured in those specimens with 36 and 48 chromosome numbers. This attribute was therefore considered to be of limited importance in distinguishing between chromosome groups. It did however, separate out three OTUs which had cleistogamous flowers much longer than the others. This attribute was also included in the numerical analyses.

2. Leaf Characteristics

The attributes most commonly enlisted to differentiate among members of the *nuttallii* complex were the characteristics of leaf size and shape: length, width, basal shape of the leaf blade, margin serration, and pubescence. With the exception of serration, these characters were easily quantified. Serration was included in the

Figure 4. Chasmogamous petal length and Cleistogamous sepal length in the *Viola nuttallii* complex.



numerical analyses as an attribute with ordered character states from entire (0) to serrate (3).

Diploid OTUs had generally narrower leaf blades, though their length was not vastly different from those of other OTUs (Figure 5). Tetraploid OTUs, on the other hand, had wider and somewhat shorter leaf blades. As for the octoploids and hexaploids, their leaf blades were wider still and some were much longer than those of the diploids and tetraploids. These two characters, although of obvious value in separating the diploids and tetraploids, were of limited utility in distinguishing between octoploids and hexaploids.

The shape of the leaf blade was described by the two attributes of basal blade angle and the length to width ratio (Figure 6). Diploid OTUs possessed leaves with truncate bases (angles approaching 180°) and blades generally twice as long as wide. Tetraploid OTUs displayed leaf blades up to five times as long as wide with attenuate bases (angles of less than 60°). Again, these two characters alone did not discriminate between the 2N = 36 and 48 chromosome levels. These OTUs commonly had leaf blades two to three times as long as broad, with cuneate (angles of 50° to 80 °) or truncate leaf bases.

The length of leaf pubescence was measured on trichomes which appeared on the leaf midrib near its base, where even near-glabrous plants often displayed some pubescence. Both groups 2N = 12 and 24 had short trichomes averaging 0.14 mm long (Figure 7). The 2N = 36 chromosome group exhibited the widest range of variability, from entirely glabrous to those with trichomes 1 mm long. The octoploids had trichomes generally 0.3 mm long, but this fell within the range of hexaploid variability.

Using the Student's t-test (Campbell, 1974), trichome length was not found to be significantly different between diploids and tetraploids, nor between hexaploids and octoploids. There were significant differences (P = 1.0%) between all pairs of combinations between the above two groups.

Because of the ability of this attribute to distinguish between at least two groups of OTUs, it was included in the numerical analysis, even though trichome length alone was not a dependable method of determining ploidy level.

Figure 5. Cauline leaf length and width in the *Viola nuttallii* complex.

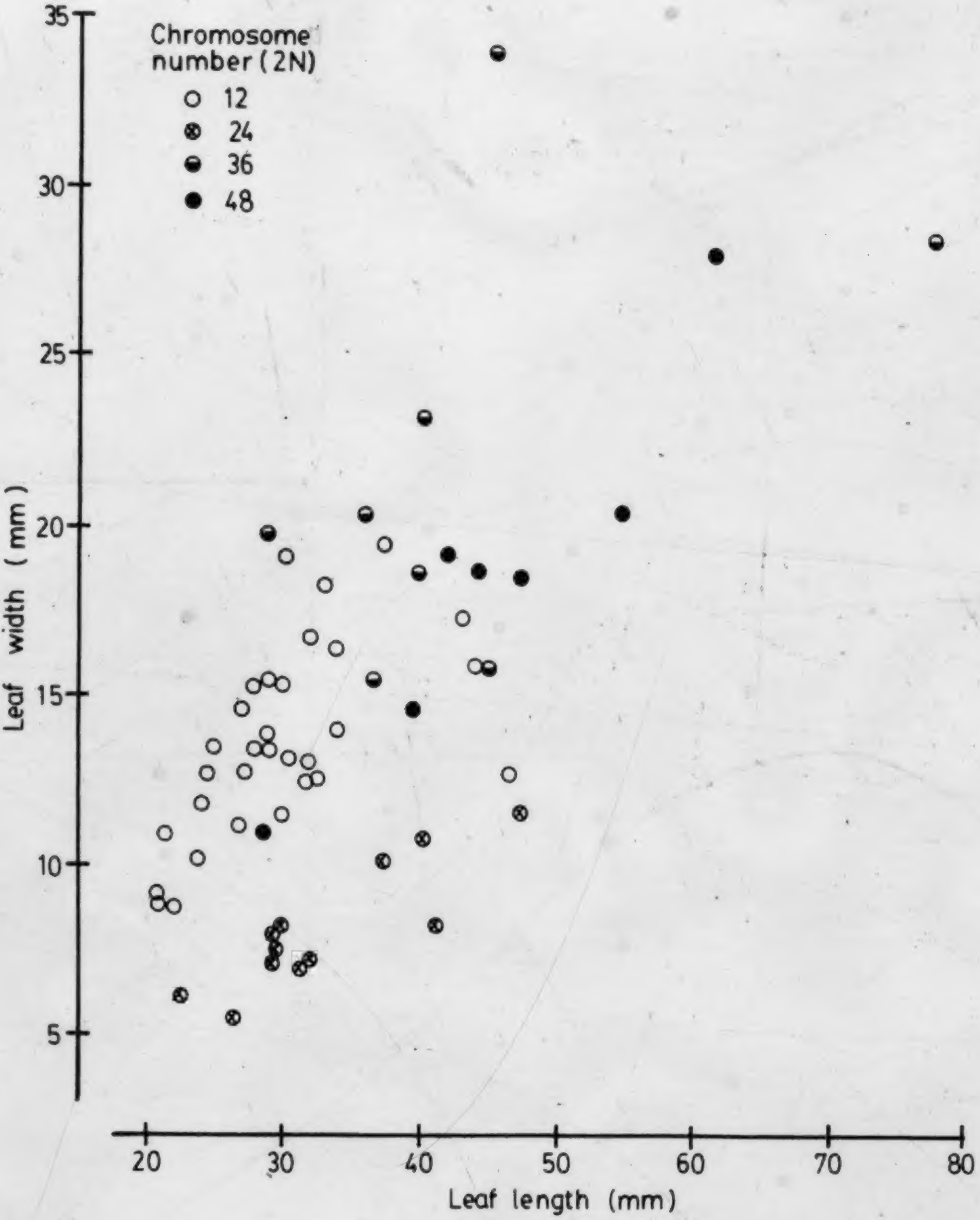


Figure 6. Cauline leaf length to width ratio and blade basal angle in the *Viola nuttallii* complex.

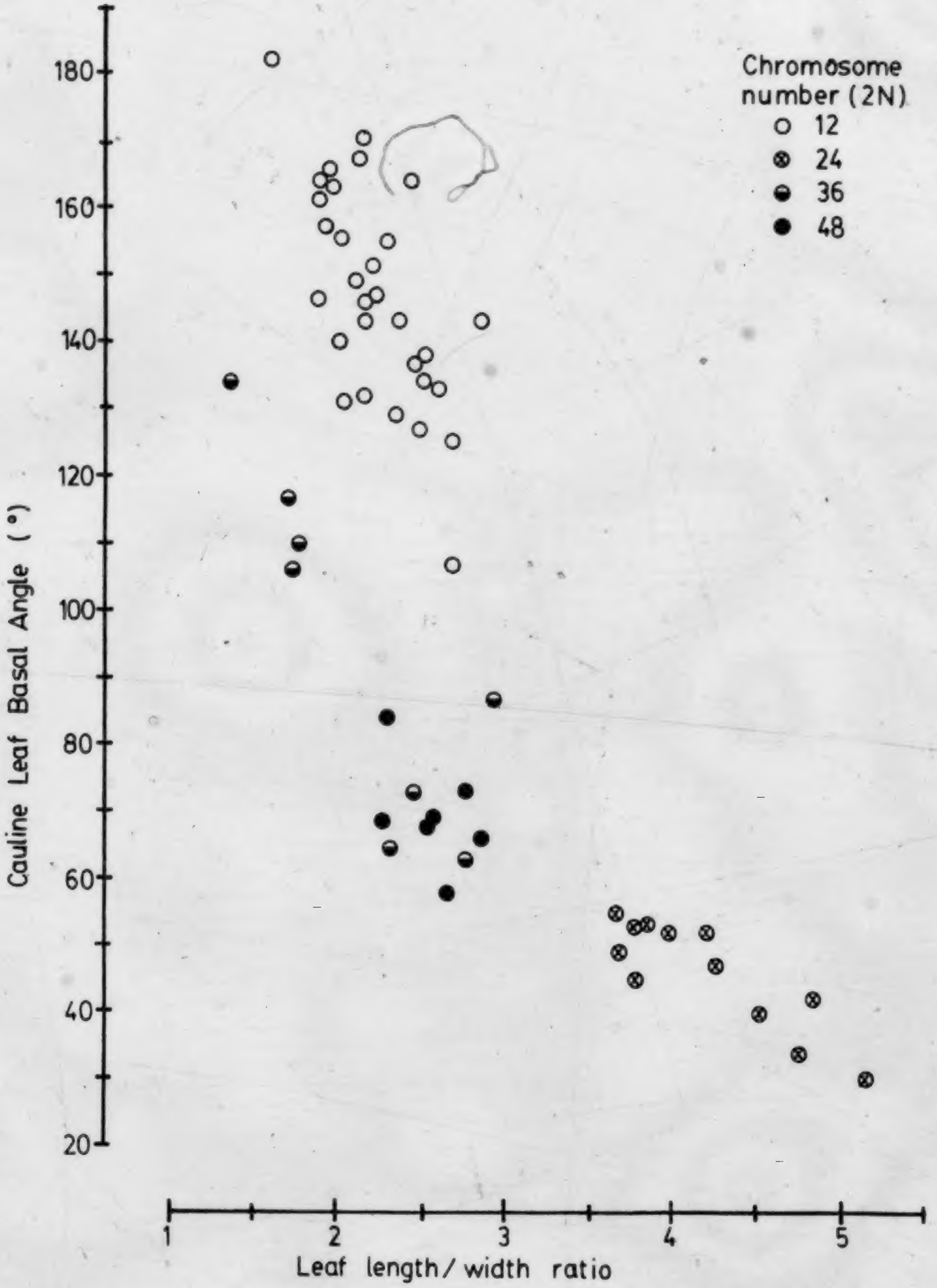
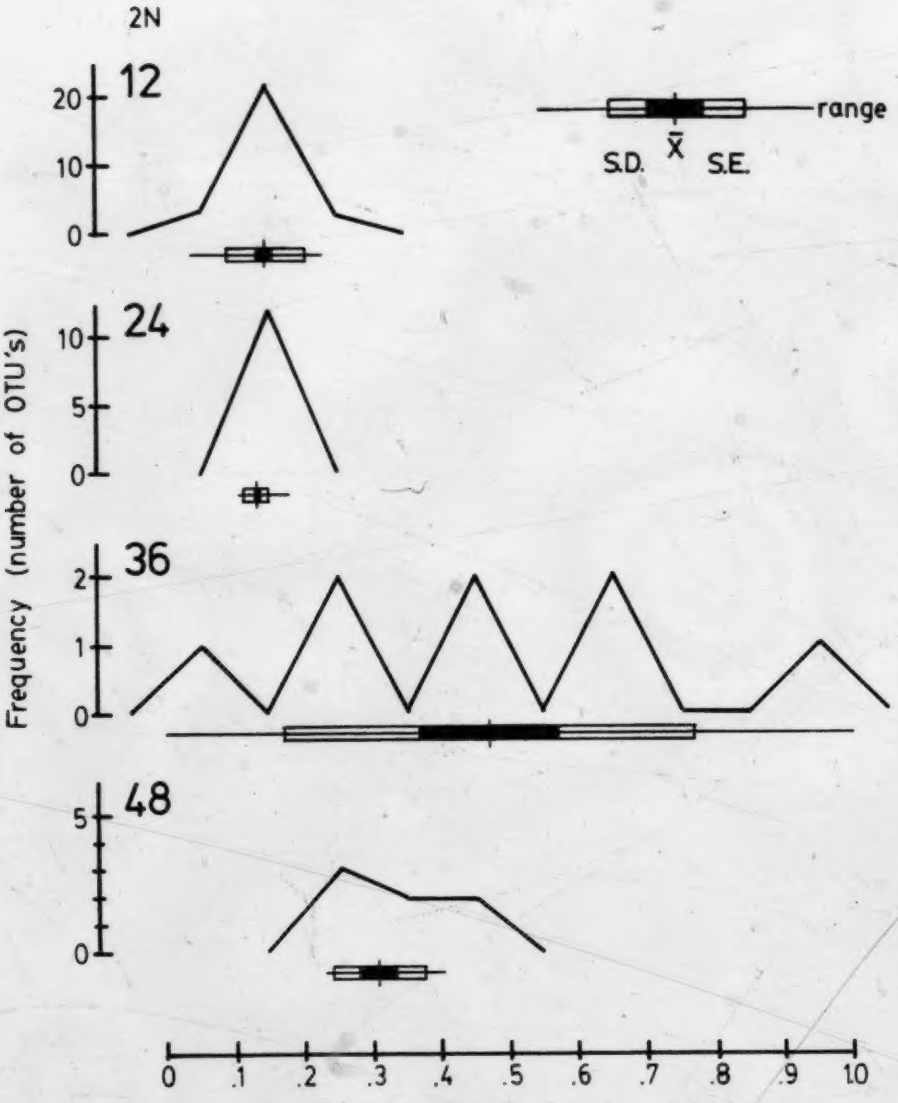


Figure 7. Leaf trichome length in chromosome races of the *Viola nuttallii* complex.



3. Seed Characteristics

Size, shape, colour, weight, and caruncle forms of seeds have been described for some taxa in the Nuttallianae (Baker, 1957, 1949b) but have not been used in distinguishing among taxa or groups of taxa.

Seeds were collected from the chasmogamous and cleistogamous fruit of living specimens as described previously. Using the paired sample t-test, no significant differences at the 0.1% level were evident between chasmogamous and cleistogamous seeds for the above attributes. No distinction was made, therefore, between the sources of seeds.

Seed length and width measurements are illustrated in Figure 8 for 58 OTUs, 55 of which were cytologically known. Diploid OTUs possessed tan coloured seeds 1.9 to 2.3 mm long and 1.1 to 1.4 mm wide. Three notable exceptions should be mentioned. All three appeared to be longer than the others and one was also wider (DF 489, 488 and 612). Two of these anomalous OTUs also displayed darker seed colouring than seen in other diploids.

Tetraploid OTUs possessed brown seeds that were both wider and longer than those of the diploids.

Hexaploid OTUs displayed a variety of seed coat colours including white, light brown, dark brown, and red-brown. Hexaploid seeds were normally longer and wider than those of the diploids and generally wider than those of the tetraploids. There appeared to be no clear distinction between hexaploids and octoploids in colour and size of seeds. Figure 8 indicates that diploids and tetraploids can be readily distinguished from each other and from the octoploids and hexaploids.

Seed length-to-width ratio ranges for all cytological types overlap, though tetraploid OTUs have slightly longer seeds and octoploids and hexaploids have generally wider seeds (Figure 9). These differences in form are complemented by differences in weight: seed weight tended to increase with chromosome number.

It was notable that two diploid OTUs (DF 488, 489) displayed characters similar to the tetraploids. These were the same OTUs which showed anomalous character states in seed length and width (Figure 8).

Figure 8. Seed length, width and coat colour in the *Viola nuttallii* complex.

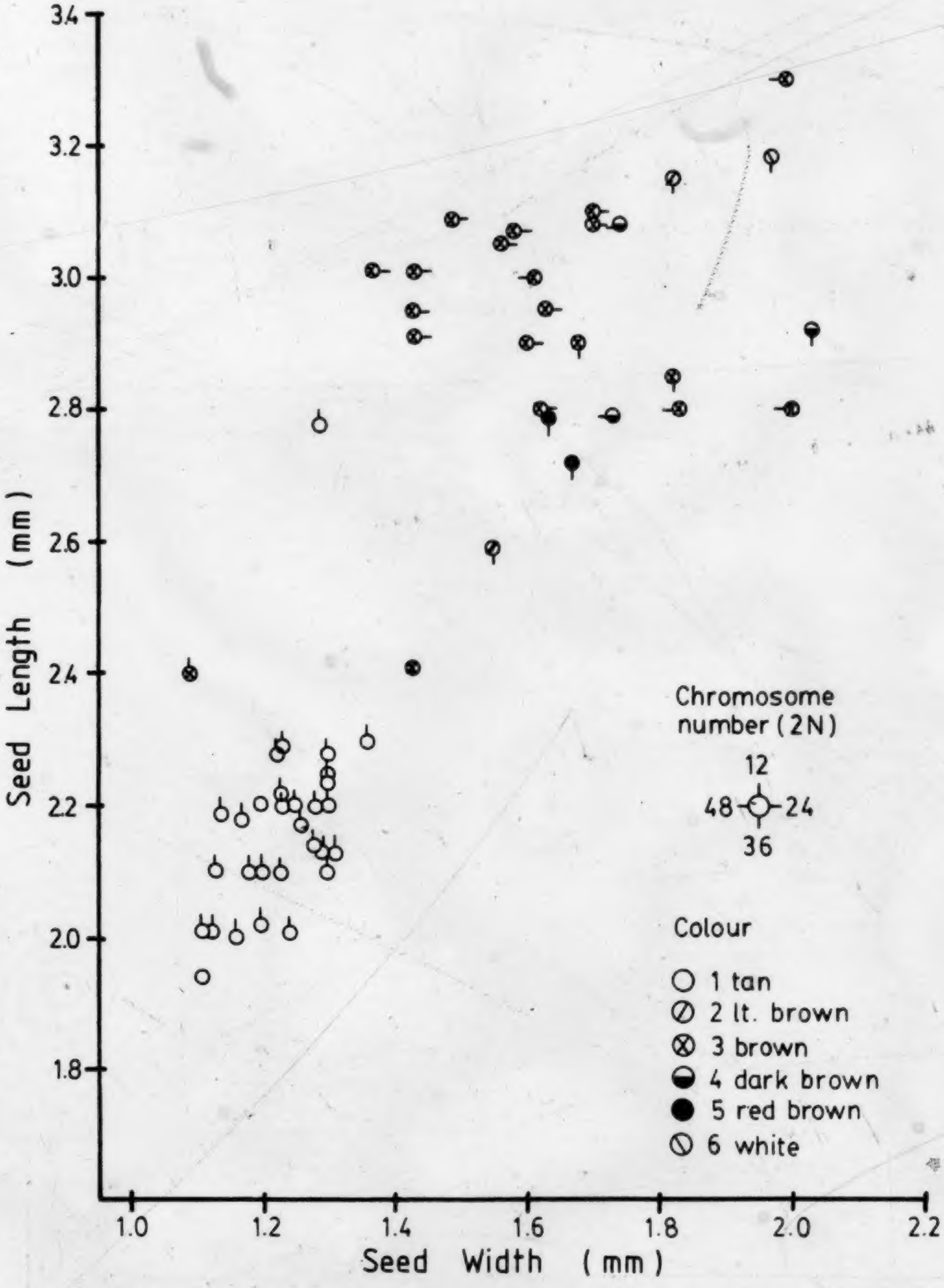
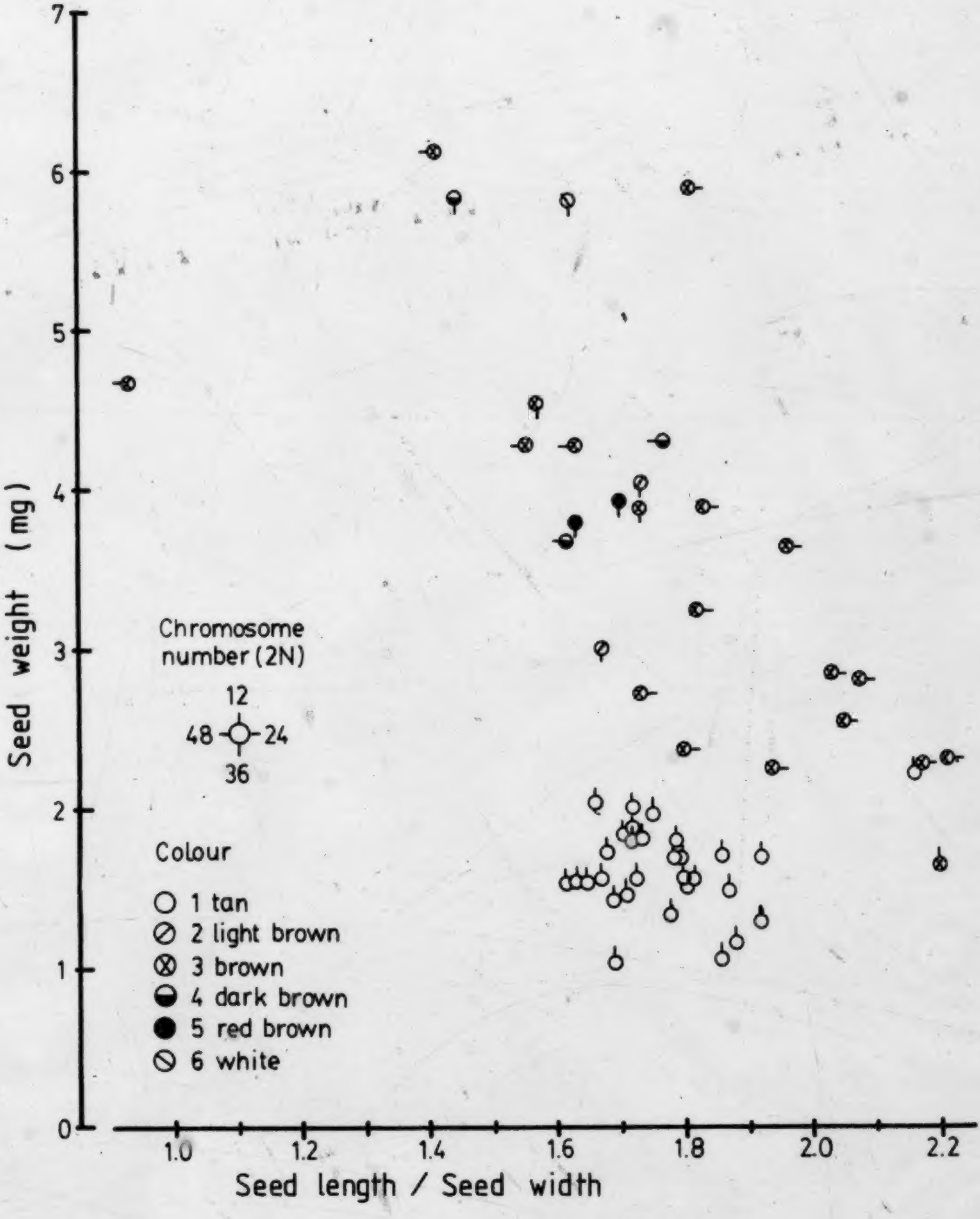


Figure 9. Seed length to width ratio, weight and coat colour in the *Viola nuttallii* complex.



Due to the clear distinction of at least three groups of OTUs by seed length to width ratio, seed weight and seed coat colour, these attributes were incorporated into the numerical analyses.

Baker (1949b) noted that the caruncle of *Viola* seeds was large enough to almost cover the entire funiculus. Through observation, four features of the caruncle seemed to be possible distinguishing features. The form of the caruncle was recorded as unordered state characters, either absent (0) or present (1). The caruncle typically was either dorsally flattened (1) or of amorphous form (0); it either extended to cover the tip of the seed (1) or was attached in a limited area and did not cover the tip (0). These two features and the total length of the caruncle and the percent of the length of the seed which it covered are illustrated in Figure 10.

Diploids possessed caruncles of 0.7 to 0.9 mm in length that extended over 29 to 40% of the seed length. Tetraploids displayed significantly (at P = 0.1) longer caruncles which extended over the same proportion of the seed length and often beyond the seed tip. Tetraploid seed caruncle length was not significantly different from either the hexaploid or octoploid seeds, though the relative length of the caruncle was significantly (P = 0.1) different from that of hexaploids.

The form of the caruncle correlated well with two cytological groups. The 2N = 12 and 24 OTUs possessed seeds with caruncles which were dorsally flattened and covered the tip of the seed, while the 2N = 36 and 48 OTUs had globular caruncles which did not extend over the tip of the seed. One notable exception was an octoploid (DF 395) with a dorsally flattened caruncle which did not extend over the tip of the seed.

C. Flavonoid Chemistry

Twenty-nine flavonoid compounds were detected in the leaf tissue of members of the *Viola nuttallii* complex including flavone glycosides, flavonol glycosides and aglycones. A composite two-dimensional paper chromatographic profile of all compounds is given in Figure 11. All available chromatographic and spectral data for these compounds is presented in Table 6. Compound numbers in Figure 11 and Table 6 correspond to attribute numbers in the numerical analysis. Equivalence of compounds was judged by colour reactions, Rf values and co-chromatography.

Figure 10. Seed caruncle length and form in the *Viola nuttallii* complex.

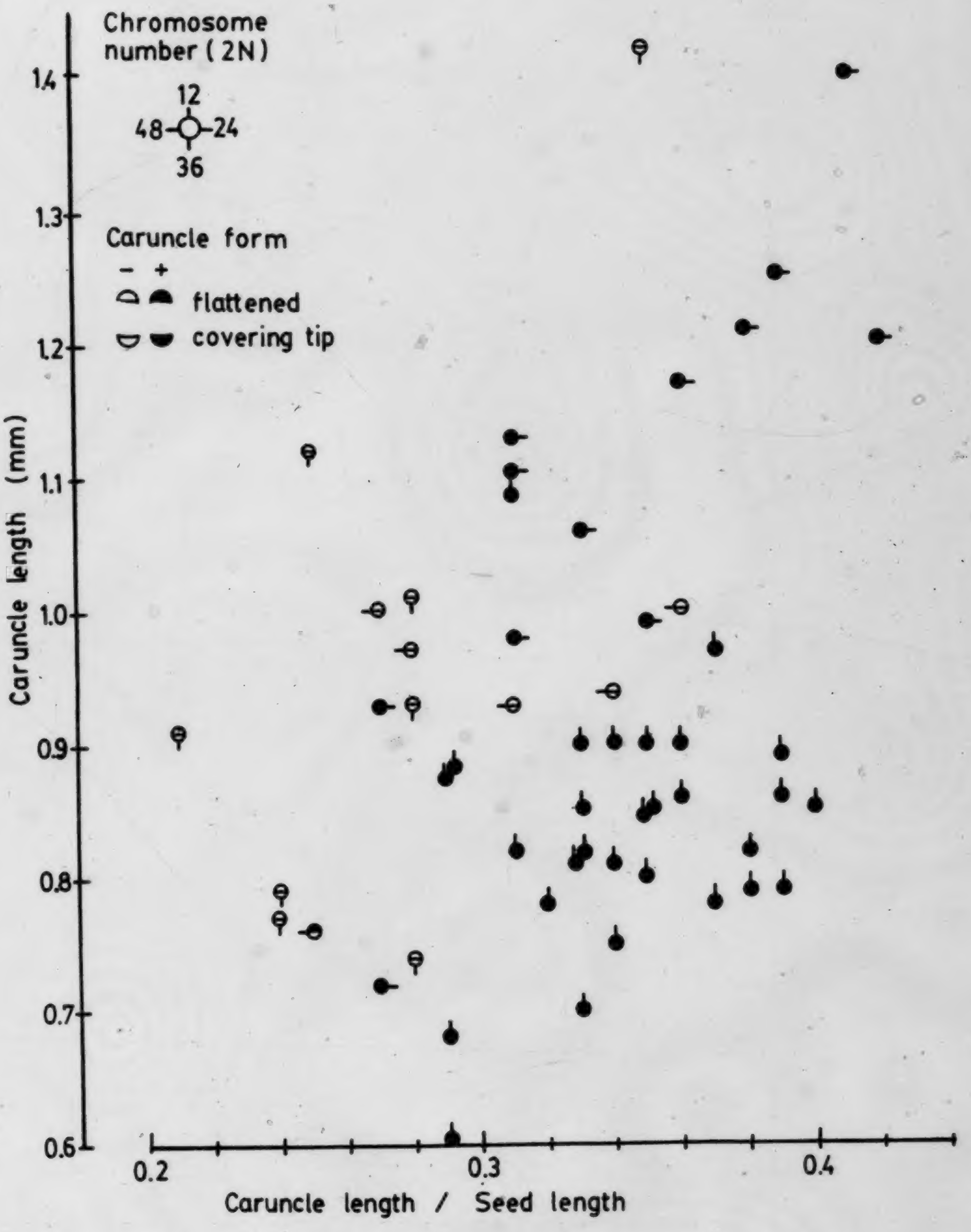


Figure 11. Composite chromatograph of the flavonoid glycosides of the *Viola nuttallii* complex. Numbers correspond to attribute numbers in the numerical analyses.

1.	**Kaempferol 3-O rut**
2.	**Apigenin 7-O rut**
3.	**Apigenin ?**
4.	**Kaempferol 6-Me, 3-O rhm,glu**
6.	**Quercetin 3-O rut**
7.	**Quercetin 3,7-O diglu**
8.	**Quercetin 3-O glu**
9.	**Kaempferol 3,7-O rhm,gal**
11.	**Luteolin 7-O gal**
13.	**Luteolin**
14.	**Apigenin 7-O glu**
15.	**Luteolin 6-Me, 7-O glu,ara**
16.	**Apigenin 7-O glu**
17.	**Luteolin 7-O triglu**
27.	**Quercetin 3-O digly**

Compounds: A= apigenin, K= kaempferol, L= luteolin, Q= quercetin, ara= arabinose, gal= galactose, glu= glucose, gly= glycoside, rhm= rhamnose, rut= rhamnosylglucoside, xyl= xylose.

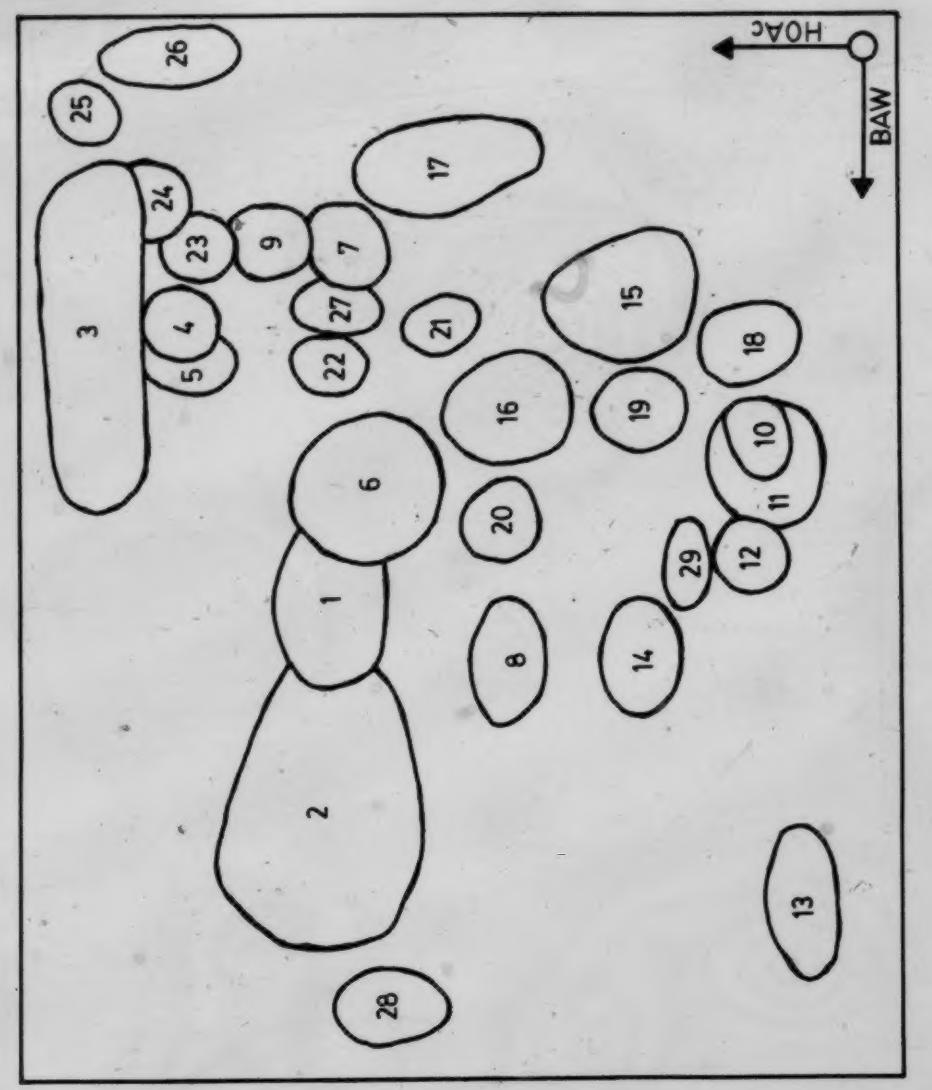

HOAc
BAW

Table 6. Chromatographic and spectral data for flavonoid compounds isolated from the leaf tissue of members of the *Viola nuttallii* complex.

NO	Colour[1]	Chromatography (Rf X 100)								Spectral Data[4]								
		Paper[2]				Thin Layer[3]				MeOH		NaM	$AlCl_3$	+HCl	NaA	H_3BO_3		
		A	B	C	D	E	F	G	H	II	I	I	I	I	I	II	I	Compound
1	p-bk-y	58	27	55	64	18	31	60	4	267	349	405	354.402	350.400	405	267	356	K 3-O rut
2	p-y-yg	71	36	72	70	18	31	60	4	267	349	383	347.402	343.399	382	266	345	A 7-O rut
3	p-g-yg	33	57	72	55	72	6	89	18	268	335	400	350	347	360	268	345	A ?
4	p-bk-bk	35	25	61	59					269	328	410	350	348.	399	268	350	K 6-C,3-O rut
5	p-g-O	36		61														
6	p-yg-yo	46	23	53	47	25	24	53	55	260	360	428	435	368.400	405	256	373	Q 3-O rut
7	p-y-o	24	38	61	40	61	6	69	15	258	360	416	440	366.406	410	262	382	Q 3,7-O diglu
8	p-y-o	62	5	52	53	6	22	23	82	256	360	430	414	366.400	425	262	382	Q 3-O monoglu
9	p-g-yg	28	44	69	53	66	6	77	12	266	350	400	352	350	400	266	350	K 3,7-O rhm,gal
10	o-y-y	32	9	44														
11	p-y-o	43	1	16	67	11	32	11	81	256	350	398	430	360.390	404	260	374	L 7-O gal
12	o-o-y	58		15														
13	p-g-y	86	0	4	59	0	29	4	97	254	348	403	420	358.386	400	252	370	L
14	p-g-og	58	5	23	67	11	31	16	83	269	329	394	383	344.380	389	269	334	A 7-O monoglu
15	p-y-yo	29	6	31	51	35	16	27	23	257	351	397	432	366.390	406	260	373	L 7-O monoglu
16	p-g-yg	37	8	39	65	34	31	35	23	270	330	392	349	344	402	269	334	A 6-C,7-O glu,ara
17	p-y-yo	26	10	38	39	45	5	35	9	258	351	401	433	366.390	403	260	373	L 7-O triglu
18	p-y-y	33		25														
19	p-y-y	38		36														
20	p-g-g	49		47														
21	p-y-bk	34		58														
22	p-yg-g	34		69														
23	p-o-o	24		77														
24	p-o-o	21		83														
25	p-o-o	11		88														
26	p-bl-blo	6		77														
27	p-yo-o	31	29	60	44	45	10	59	20	258	357	431	406	362.398	410	262	380	Q 3-O digly
28	p-bl-bl	95		63														
29	o-w-y	51		15														

[1] Colours: UV - UV+NH_3 - UV+NA; bk= black, bl= blue, g= green, o= orange, p= purple, w= white, y= yellow.
[2] Paper Chromatography: A= BAW, B= H_2O, C= 15% Acetic acid, D= Phenol.
[3] TLC: E= Polyamide-aqueous, F= Polyamide-organic, G= Cellulose, H= Silica.
[4] Spectra: 100% MeOH, NaOMe (NaM), $AlCl_3$, $AlCl_3$ + HCl, NaOAc (NaA), H_3BO_3, I = Band I, II = Band II,
[5] Compounds: A= Apigenin, K= Kaempferol, L= Luteolin, O= Quercetin, ara= arabinose, gal= galactose, glu= glucose, rhm= rhamnose, rut= rutin, gly= glycoside, ?= glycosylation unknown.

Of the 83 OTUs extracted for flavonoid analysis, 79 were cytologically known, 24 of these were specimens taken from herbarium sheets on loan from DS, CAS, UC and MO (Table 4).

Three flavonoid patterns were discovered within the diploid (2N = 12) chromosome level (Table 7). Groups 1, 2 and 3, though not morphologically distinct, were distinguished by different chromatographic patterns. In group 1, the major flavonoids 2 and 3 were both apigenin glycosides. Group 3 OTUs contained 1, 9, 10 and 11, kaempferol and luteolin glycosides. Though not consistently separable by means of any of the morphological features examined previously, groups 1 and 3 were found to be geographically separated, occuring east and west of the Rocky Mountains, respectively. Group 2 consisted of six specimens from the Great Basin region, that had intermediate flavonoid profiles. They contained compounds in common with group 1 (1, 2 and 3) and group 2 (1, 5 and 10), and the unique compound, 29.

Group 4, also 2N = 12, consisted of one specimen of *V. tomentosa* (*M. S. Baker 8699*, OTU 83) which possessed attributes 1, 2, 3, 6, 7, and 8, apigenin, kaempferol and quercetin glycosides.

The OTUs containing 24 somatic chromosomes (group 5) bore some similarity to groups 1, 2, 3 and 4 in possessing attributes 1, 3, 4, 6, and 7. These compounds correspond to an apigenin glycoside, kaempferol 3-0 diglycosides and quercetin 3-0 and 3,7-0 diglycosides.

The eight compounds mentioned thus far were found consistently in over 50% of the OTUs in a given group. The groups contained from 4 to 8 different flavonoid compounds each.

A much greater variety of flavonoid compounds was found in the remaining groups, those with chromosome complements of 36 and 48. These were segregated in Table 7 according to preliminary taxonomic groupings based on identifications made by Baker and Clausen: 6, *praemorsa*; 7, *oregona*; 8, *major*; 9, *arida*; 10, *bakeri*; and, 11, *linguaefolia*.

Group 11 was the only group with a distinct flavonoid profile. Like groups 1 to 5, it possessed compounds 1 and 10; like groups 5 to 10 it contained compounds 6, 7, 8 and 9; and unique to this group was compound 27. Group 11 contained a variety of

Table 7. Distribution of flavonoid compounds in the *Viola nuttallii* complex.

	Frequency (X100)										
Taxon[1] Attribute[2]	1	2	3	4	5	6	7	8	9	10	11
2 A 7-O digly	96	17		100							
3 A -	100	100		100	100						
14 A 7-O monogly						100	50	64	40	100	
16 A 6-C,7-O gly						100	100	73	80	86	
13 L						100	50	55	80	86	
11 L 7-O monogly			17			100	100	100	80	100	
15 L 7-O gly						100	100	91	100	100	
17 L 7-O trigly						100	100	73	100	100	
1 K 3-O digly	4	100	100	100	55						100
4 K 3-O digly	13				73						
9 K 3,7-O gly		100				66	100	55	80	86	75
6 Q 3-O digly	4		100	100	100		100	73	100	43	100
8 Q 3-O monogly				100	27	33	50	45		14	50
7 Q 3,7-O gly	9			100	82	66	50	27	80	43	100
27 Q 3-O trigly											75
5 -		100									
10 -		50	17		9						25
12 -					9						
18 -								9			
19 -						33		9	40		
20 -								18	20		
21 -							18			29	
22 -						33	100	18	40	14	
23 -								18		29	
24 -				100				18	40	14	
25 -								45	20	43	
26 -						33	50	9		14	
28 -									60	43	
29 -			16					18			
Total flavonoid attributes / taxon	5	6	4	7	8	13	12	20	15	17	7
Chromosome No.	12	12	12	12	24	36	36,48	36,48	48	48	48
Total populations surveyed	23	6	6	1	11	3	2	11	5	7	4

[1] Taxa: 1 = *vallicola*, 2 = *vallicola*, 3 = *vallicola*, 4 = *tomentosa*, 5 = *nuttallii* 6 = *praemorsa*, 7 = *oregona*, 8 = *major*, 9 = *arida*, 10 = *bakeri*, 11 = *linguaefolia*.

[2] Compounds: A = Apigenin, K = Kaempferol, L = Luteolin, Q = Quercetin, gly = glycoside.

flavonols, but no identified flavones: kaempferol 3-0 and 3,7-0 diglycosides, quercetin 3-0 and 3,7-0 diglycosides and a quercetin 3-0 triglycoside.

Compounds 6, 7, 9, 11, 13, 14, 15, 16 and 17 were commonly found in many of the OTUs in most all of the above groups. These major compounds were apigenin, kaempferol, quercetin and luteolin derivatives. None of the groups, even group 10, *bakeri*, which was morphologically distinct from the others displayed obvious, unique profiles. With increasing chromosome number, OTUs possessed increased numbers of more complex flavonols and flavones.

All raw data presented above and used in the numerical analyses are presented in Appendix 1.

D. Numerical Analysis

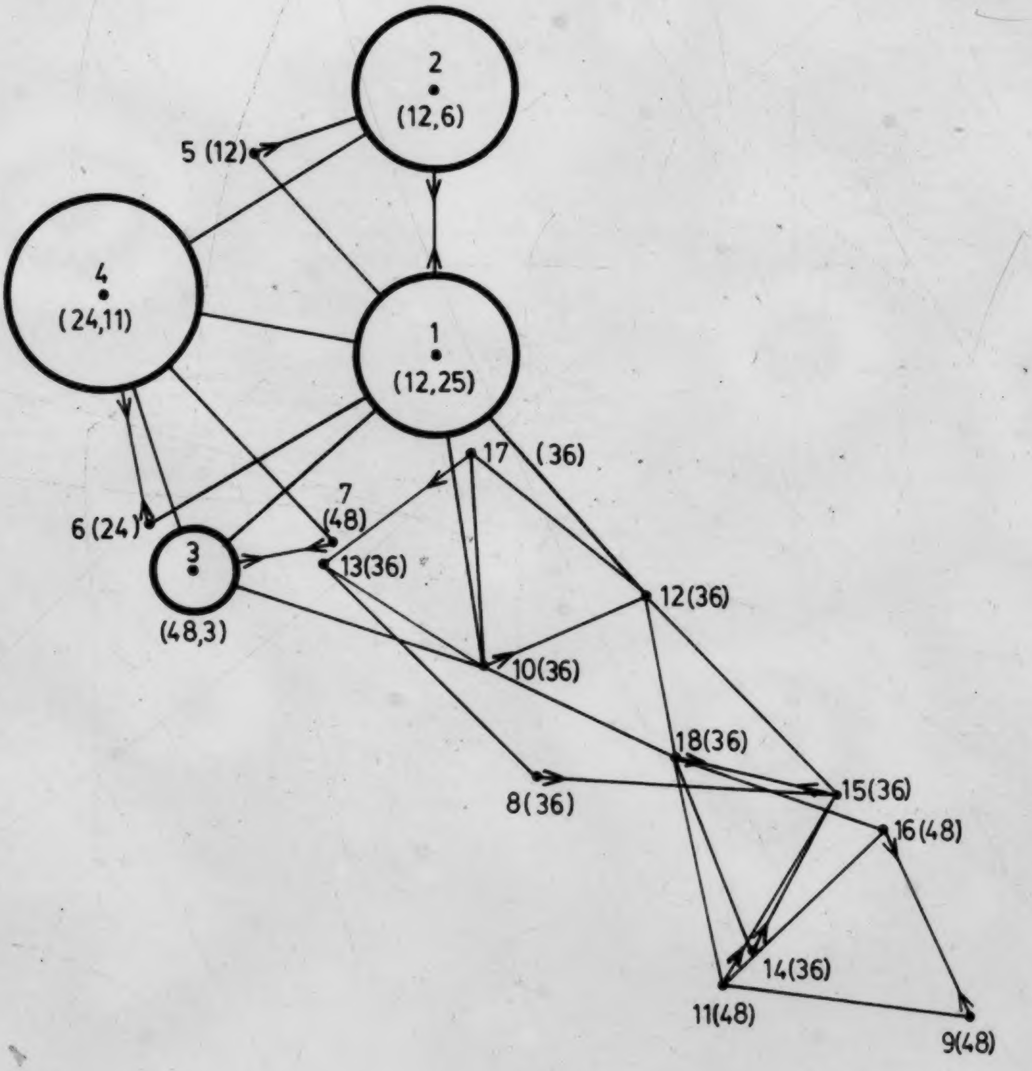
1. TAXMAP analysis one

The initial TAXMAP analysis was performed using the author's collections, which totaled 59 OTUs scored for 50 attributes, both structural and chemical in nature. Due to the maximum information content of this analysis, it was presumed to be the most accurate. The resulting clusters from the equally weighted analysis are illustrated in the CALCOMP plot of cluster configurations (Figure 12, see Appendix 2 for the complete TAXMAP printout). This analysis provided 4 multi-membered clusters and 14 single membered clusters.

Cluster 1 included 25 OTUs, all of which were found to have somatic chromosome numbers of 12. All members were located in Alberta and Saskatchewan, east of the Rocky Mountains. The flavonoids present in members of this group were attribute numbers 2 and 3 (apigenin derivatives). These were plants with small (30 mm) ovate or ovate-oblong, nearly entire leaves with truncate bases, covered with very short puberulence, possessing small flowers, and with pollen 31 um in diameter.

Closely linked to Cluster 1 was Cluster 2 which contained morphologically similar OTUs but which had additional flavonoids: attributes 1 and 9 (kaempferol 3-O diglycoside and kaempferol 3,7-O glycoside, respectively). All six members of Cluster 2 were collected in British Columbia or Oregon, west of the Rocky Mountains.

Figure 12. TAXMAP cluster analysis of 59 OTUs for 50 attributes, equally weighted analysis. Dots indicate cluster centers, circles indicate multi-membered cluster diameters, arrows indicate nearest neighbours, and lines indicate intercluster distances. Each cluster is defined by its cluster number and the chromosome number of its members in parenthesis. For multimembered clusters, the number of OTUs is also indicated in parenthesis.


2
(12,6)
5 (12)
4
(24,11)
1
(12,25)
17 (36)
7
(48)
6 (24)
3
(48,3)
13(36)
12(36)
10(36)
18(36)
8(36)
15(36)
16(48)
14(36)
11(48)
9(48)

Cluster 3 consisted of three OTUs possessing flavonoids 1, 6, 7, 8, and 9 (kaempferol and quercetin derivatives). These OTUs had a chromosome count of 48. Their leaves were generally twice as long as wide and about as wide as those in cluster 1 and 2; leaves had more cuneate bases; and, leaf pubescence tended to be slightly longer, about 0.15 mm. Seed morphology also differentiated this group. Cluster 7 (OTU 35) was the mutual nearest neighbour of cluster 3.

Cluster 4 contained 11 OTUs, all having chromosome complements of 24. These OTUs were characterized by their narrow cauline leaves which were more than three times longer than wide and had attenuate bases. Seeds of this group were the largest found in the complex and had brown seed coats. OTUs in cluster 4 commonly possessed flavonoid attributes 3, 4, 6, and 7 (apigenin, kaempferol and quercetin derivatives). Cluster 6 (OTU 29) was a single-membered cluster whose nearest neighbours were all in Cluster 4, and thus it could be considered part of it.

Clusters 8 to 18 were single-membered clusters. None of their nearest neighbours were closer than 0.15 units, unlike the distance relationships within other clusters. A more extensive analysis of these OTUs (analysis three), which included more OTUs in an attempt to overcome the problem of restricted sample size, was performed.

2. TAXMAP Analysis Two

Analysis two contained 72 OTUs and is presented in Appendix 3. The additional 13 OTUs represented type specimens (holotypes, paratypes, and isotypes) on loan from other herbaria. Due to the nature of these specimens, limited information could be obtained from them. None of these OTUs were sampled for flavonoid analysis, only three were cytologically known, and none possessed mature seeds for measurement. It must also be recognized when comparing the results of analyses one and two that since distance calculations in TAXMAP represent relative distances, cluster membership was expected to deviate somewhat from that in analysis one.

Whereas analysis one had split violets with 2N = 12 into two clusters, analysis two created nine clusters (1, 2, 4, 5, 7, 8, 9, 10, 11, and 26). which were linked or had each other as their eight closest neighbours and very small intervening distances. Membership in some of these clusters was made up of members of both cluster 1 and 2 of analysis one. For these reasons it seemed most reliable to consider all of these OTUs as one cluster.

The group with 2N = 12 now contained four type OTUs: AN4340 (*vallicola* A. Nels.); S8530 (*subsagittifolia* Suks.); RS-BB (*russellii* B. Boivin); and CFB67 (*physalodes* Greene). Single-membered cluster HOOK / K (type specimen of *major* Hooker) had four of its nearest neighbours in this group and was therefore included.

Although the type *major* Hook. clustered here and is the oldest published name associated with this large group, it was not adopted as it has no priority outside the varietal level. Nuttall collected this specimen on the banks of the Columbia River, west of the Rocky Mountains. It was appropriate to apply this name to the chemical form (cluster 2, analysis one) found there. The flavonoid form most common east of the continental divide will be designated *vallicola*, the most elligible and applicable specific name.

Cluster 6 contained OTUs with 2N = 24 chromosomes and the same OTUs as cluster 4 of analysis one. Although not a member of it, OTU ANSPH (the type specimen of *nuttallii* Pursh), single member cluster 27, was associated with this cluster by virtue of its nearest neigbour list and its intercluster distances. This specimen is more closely aligned with members of cluster 6 than with any other cluster or OTU. The members of this group shall, therefore, be refered to as *nuttallii*. As no other specimen could be associated with this group, the name is confidently applied.

Cluster 3 of analysis 2 corresponded exactly to cluster 3 of analysis one, containing OTUs of chromosome number 48. It also contained two type OTUs: CFB225, *gomphopetala* Greene and AN5481, *erectifolia* A. Nels. No type specimen of *linguaefolia* Nutt. was available for numerical analysis, but the description and distribution of this taxon indicate that it may be refered here. Close examination of the photographs of the type specimen (PH and G) indicate that based on leaf shape and pubescence as well as its location near the sources of the Oregon [River], it should be included with this cluster. Cluster 3 was assigned the oldest applicable name, *linguaefolia*.

As in analysis one, several OTUs represented single-member clusters.

3. TAXMAP Analysis Three

In an attempt to clarify the relationship between the single-membered clusters, a number of additional OTUs from herbarium loans were added to the analysis. These were chosen by virtue of the availability of chromosome counts. Permission was obtained to remove one leaf from each of these specimens for flavonoid extraction. All specimens from clusters 1, 2 and 4 of analysis one, their associated single membered clusters, and associated type specimens were removed from this analysis. Cluster three members were retained for analysis because a number of morphological attributes and chromosome counts of 2N = 48 indicated that its members were more similar to the remaining OTUs eventhough their flavonoid profiles were distinctly different. Analysis three thus contained 39 OTUs, 17 were from the author's collection, 16 from herbarium loans, and eight were type specimens.

The weighted cluster analysis is illustrated in Figure 13 (see Appendix 4 for a complete listing of the TAXMAP analysis). Analysis three produced 10 multi-membered clusters and 9 single-membered clusters.

Cluster one contained the same OTUs as were found in cluster three of analysis two: *linguaefolia* , 2N = 48.

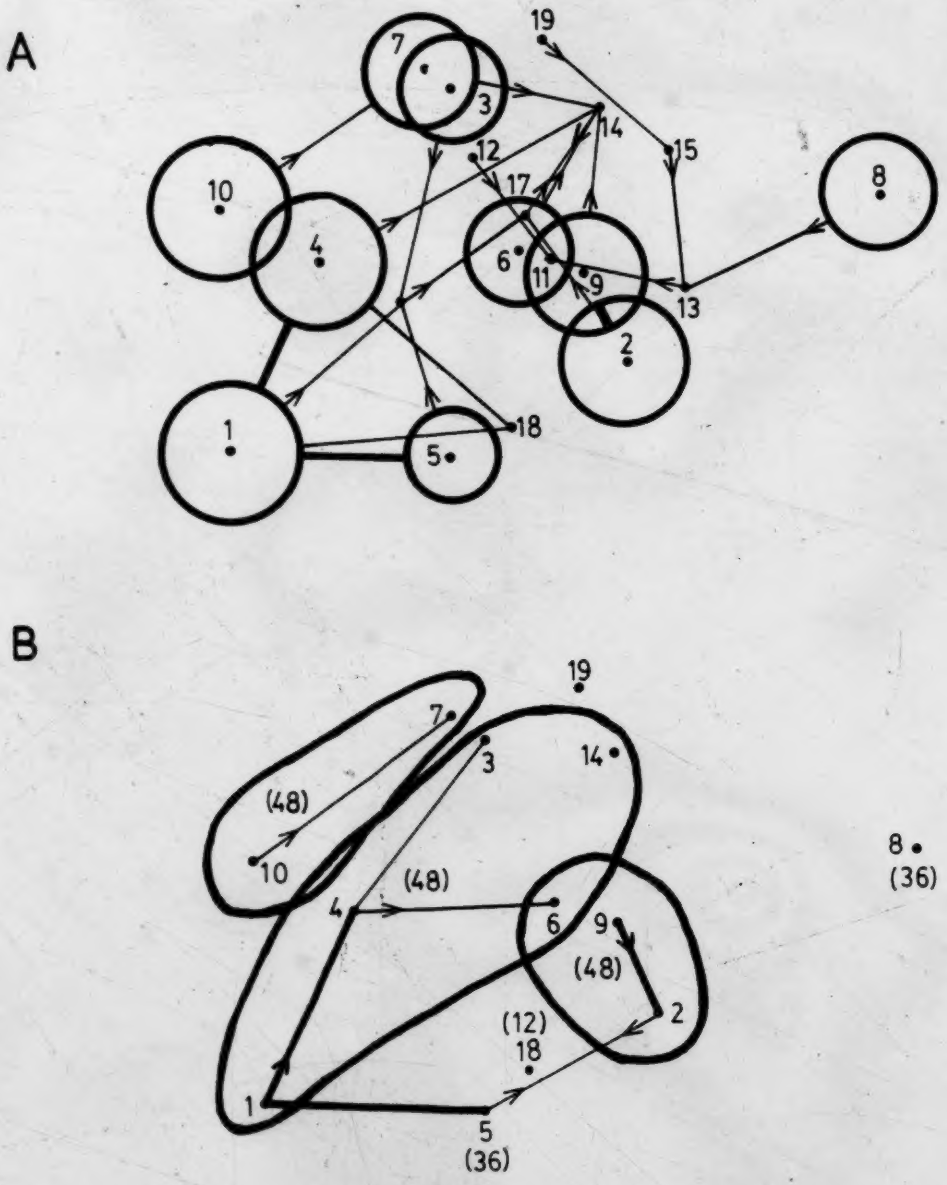
Linked to 1 was cluster 4, 2N = 48, containing the type specimen *oregona* Baker. Cluster 4 differed from one in its lack of flavonoid 1 and the presence of flavonoids 6, 7, 8, 11, 13, 14, 15, 16, 17, and 24 (quercetin, luteolin and apigenin glycosides). These OTUs had shorter leaves only 2.2 times longer than wide, with a basal angle larger than 90°, slightly longer leaf pubescence and some sepal pubescence.

Cluster 5 was also linked to cluster 1, though its members' chromosome complement was 2N = 36. Cluster 5 had no associated type specimen. It differed from clusters 1 and 4 in not containing flavonoids 7, 8 or 24 (quercetin 3-O and 3,7-O glycosides) but otherwise containing all of those found in *oregona*. Leaves in cluster 5 OTUs were slightly longer than wide (ratio of 2.5), had a smaller basal angle than *oregona*, entire margins, no sepal pubescence, seed caruncles were shorter than in *linguaefolia* with red-brown seed coats. In keeping with its chromosome complement of 36, pollen diameter in cluster 5 was smaller than that of *linguaefolia*.

Figure 13. TAXMAP cluster analysis 3, of 79 OTUs for 50 attributes in a weighted analysis.

A. Calcomp plot of cluster analysis.

B. Nearest neighbour analysis with clusters (dots) joined to nearest neighbours (arrows) or linked cluster (heavy lines) and grouped according to TAXMAP analysis using only morphological characters (heavy circles). Cluster numbers correspond to A, chromosome numbers are included in parenthesis.


A
19
7
3
14
15
12
17
10
4
6
11
9
13
8
2
1
5
18
B
19
7
3
14
(48)
10
4
(48)
6
9
(48)
8
(36)
(12)
18
2
1
5
(36)

Cluster 2 members contained flavonoids 7, 9, 11, 13, 14, 15, 16, 17, and 26 (quercetin 3,7-O and kaempferol 3,7-O glycosides), and a variety of luteolin and apigenin 7-O derivatives). These OTUs had small leaves with a length to width ratio of 2.3, a basal angle of 85°, entire margins, chasmogamous flower petals 10 mm long, glabrous capsules, and a chromosome complement of 2N = 48. These OTUs were identified as *Viola bakeri* Greene by M. S. Baker and J. Clausen on annotation labels.

Cluster 9 was linked to cluster 2 in containing basically the same flavonoids and the same chromosome number, but had slightly smaller leaves and larger cleistogamous and chasmogamous flowers. These specimens were also identified as *bakeri*.

Cluster 6 contained two OTUs with chromosome numbers of 2N = 48 and with similar flavonoid profiles to clusters 2 and 9. Morphologically similar to clusters 2 and 9, cluster 6 had only slightly larger chasmogamous petals to distinguish it from the others.

Cluster 3 contained the two isotype specimens for *arida* Baker, which have chromosome numbers of 2N =48. These specimens contained flavonoids 6, 7, 9, 11, 13, 15, 16, 17, and 28 (quercetin, kaempferol, luteolin and apigenin derivatives). Their leaves were 2.0 times as long as wide, 42 mm by 22 mm on the average, a relatively wide basal angle of 110°, very short pubescence, serrulate leaf margins, and sepals with pubescent auricles.

Cluster 7 contained only two OTUs with chromosome numbers of 2N=48 and flavonoids 6, 14, 15, 16, 17, and 24 (Quercetin, Apigenin, and Luteolin derivatives). Their ovate-oblong leaves were large, 66 by 30 mm, had cuneate bases, were pubescent, serrate, and had large chasmogamous flowers.

Cluster 7 was the nearest neighbour of cluster 10 which contained the two type specimens of *flavovirens* Pollard and one OTU with a chromosome count of 36. Most morphological features were very similar though the size of leaves in cluster 10 OTUs were slightly larger. The OTUs in this cluster will be refered to as *flavovirens*.

Part B of Figure 13 presents a simplification of Part A with most single-membered clusters removed, except those which were type specimens. Clusters were represented only by their centers, linkages between clusters were represented by thick lines, and nearest neighbours were represented by lines with directional arrows. Interpretation of the nearest neighbour indexis is cautioned as the intercluster distances represented here were relatively large (greater than .250) and do not vary much at all from intracluster OTU distances.

When TAXMAP analysis three was run using only morphological attributes (30 to 50), the clusters of analysis three were reduced in number (Appendix 4). Figure 13 Part B illustrates the new clusters by circling and linkages between these new clusters was indicated by overlapping the circles. Clusters 1, 3, 4, 6 and 14 were grouped togeather (*linguaefolia*, *arida*, *oregona*, *bakeri*, and *arida*, respectively). Clusters 2 and 9 (*bakeri*) were also closely linked to the new larger cluster. Cluster 8 (*praemorsa*) and cluster 5 (triploid) remained intact. Two members of clusters 7 and 10 were clustered together while the other OTUs previously in these clusters were found in single-membered clusters (*flavovirens*).

Cluster 19 (type *xylorrhiza*) was noteworthy by remaining as a single-membered cluster and not in close association with any other cluster. Cluster 18 (*tomentosa*) also remained as a single-membered cluster, though this was not as surprising considering its distinctive features of chromosome number (2N = 12) and very dense tomentose pubescence. This OTU contained the flavonoid constituents 1, 2, 3, 6, 7, 8 and 23 (apigenin, kaempferol, and quercetin derivatives), a range of compounds intermediate to those found in *nuttallii* and *linguaefolia*.

4. Kruskal-Wallis Test

The Kruskal-Wallis test is a nonparametric analysis of rank which does not require the assumptions of normality and equal variances in randomly sampled populations having continuous distributions. This test was employed to test the null hypothesis that the distribution of an attribute was the same in all strata, or clusters, against the alternate hypothesis that at least one population differed from the others.

Table 8 lists all 50 attributes and their significance level based on the Kruskal-Wallis test on eight TAXMAP clusters designated in analysis one and three. The characters were divided into three significance levels. Seventeen attributes including one structual attribute, were not significant at the 5 percent level and were therefore considered invalid for separating taxa. Of the remaining thirty-three attributes, twenty-eight were significant at less than or equal to the 1 percent level. It was attributes in this latter

Table 8. Kruskal-Wallis test for significance of flavonoid and morphological attributes.

Not significant at .05 level	Significant at .05 level	Significant at .01 level
Flavonoid Attributes[1]		
5 -	14 A 7-O monoglu	1 K 3-O rut
8 Q 3-O glu	16 A 6-C,7-O glu,ara	2 A 7-O rut
10 -		3 A
12 -		4 K 6-C,3-O rut
18 -		6 Q 3-O rut
19 -		7 Q 3,7-O diglu
20 -		9 K 3,7-O rha,glu
21 -		11 L 7-O monogal
22 -		13 L
23 -		15 L 7-O monoglu
24 -		17 L 7-O triglu
25		
26		
27 Q 3-O digly		
28		
29		
Cytological and Morphological Attributes[2]		
37 leaf margin ciliation	38 CH sepal pubescence	30 Ploidy level
	40 CL sepal length	31 Cauline leaf length
	41 capsule pubescence	32 Cauline leaf width
		33 Leaf length / width
		34 Leaf basal angle
		35 Pubescence length
		36 Leaf margin serration
		39 CH petal length
		42 Seed length
		43 Seed width
		44 Seed length / width
		45 Seed weight
		46 Caruncle length
		47 Caruncle / seed length
		48 Caruncle covering tip
		49 Seed colour
		50 Pollen diameter

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[1] Compounds: A = Apigenin, K = Kaempferol, L = Luteolin, Q = Quercetin, gly = glycoside, ara = arabinose, gal = galactose, glu = glucose, rha = rhamnose, rut = rutin.

[2] CH = chasmogamous, CL = cleistogamous.

group which were considered the most reliable for separating taxa, and were used in making the taxonomic keys.

E. Taxonomy

Viola L. (Sp. Plant. Ed. I:933. 1753.)

Small trees, shrubs or herbs, the latter annual or perennial; leaves simple, alternate or basal with foliaceous stipules; flowers perfect, zygomorphic, on axillary, one-flowered peduncles, often inverted; chasmogamous flowers, petaliferous, pentamerous and irregular as to calyx, corolla, and stamens; sepals 5, auricled, persistant; petals 5, lowest spurred or saccate, others in two pairs, the laterals usually bearded within, imbricate on the bud; stamens closely surrounding the ovary, distinct but more or less coherent, two lower with nectar bearing appendages projecting into the spur, filaments short and broad or lacking; ovary hypogynous, pistil solitary, style one, with usually oblique stigma, unilocular with three parietal placentae; fruit a loculicidal capsule, ovoid to cylindric, cartilaginous, valves three, seminiferous in middle, contracting when open and ejecting seeds; seeds ovoid, anatropous with a crustaceous coat, carunculate; embryo straight in copious endosperm, cotyledons flat; cleistogamous flowers, if present, with petals rudimentary or lacking, self-fertilizing within the closed calyx, occasionally subterraneous.

Type species of the genus: *Viola odorata* L.

Key to the species of the *Viola nuttallii* complex:

1. a. Leaves entire or nearly so, minutely puberulent, hairs 0.01 to 0.15 mm long, or glabrous; seeds tan or medium brown; caruncle flattened, extending beyond and covering the funiculus.

  2. a. Leaves more than 3.5 times longer than wide, 6 to 13 mm wide, base attenuate (angle 30 to 55°); seeds medium brown, 3mm long and 1.5 mm wide; pollen 35.2 um in diameter; found only east of the Rocky Mountains at elevations of 700 to 2400 m; 2N=24 . . . . *V. nuttallii*

2. b. Leaves 2.0 times longer than wide, base truncate (angle 120 to 180°), some later cauline leaves becoming cuneate; seeds mostly tan, 2.2 mm long and 1.2 mm wide; pollen 31 um in diameter; found from Great Basin to mid-western plains at elevations of 400 to 2800 m; 2N = 12 . . . . *V. vallicola*

1. b. Leaves entire, serrulate or serrate; pubescent throughout, hairs greater than 0.5 mm long, or occasionaly glabrous; seeds silvery-purple to glossy reddish-brown; the caruncle globular, not extending beyond or covering the funiculus.

3. a. Cauline leaves 13.5 mm wide and more 2.3 to 2.5 times longer than wide, margins entire; chasmogamous flowers 8 to 15 mm long; seeds 1.7 to 1.8 mm wide, reddish brown; 2N = 12 or 48

4. a. Densely tomentose throughout, hair length 1.4 mm or longer; seeds 2.7 mm long and 2.0 mm wide, 1.4 times longer than wide, caruncle small, 0.5 mm; 2N = 12 . . . . *V. tomentosa*

4. b. Densely pubescent throughout, hair length 0.3 to 0.4 mm; seeds 3.1 mm long and 1.8 mm wide , 1.7 times longer than wide, caruncle 0.9 mm long; 2N = 48 . . . . *V. bakeri*

3. b. Cauline leaves 14 to 30 mm wide and 1.6 to 2.6 times longer than wide, margins crenate or serrate; chasmogamous flowers 12 to 20 mm long; seeds 1.7 to 2.0 mm wide, medium to dark brown; 2N = 36 or 48 . . . . *V. praemorsa*

Viola nuttallii Pursh. Fl. Am. Sept., 1: 74. 1814.

Type: "On the banks of the Missouri. June. v.s. Herb. Nuttall." PH! (holotype).

Crocien nuttallii (Pursh) Nieuwland and Lunell. Am. Midl. Nat. 4(11): 478. 1916.

A herbaceous perennial, 5 to 20 cm tall, from an erect, woody rootstock; glabrous or with puberulence throughout or only on leaf veins; stem appearing acaulescent or becoming greatly elongated especially in late season growth; radical leaves ovate to oblong-lanceolate, acute, cuneate to attenuate ; cauline leaves mostly lanceolate, 26 to 40 mm long, 6 to 10 mm wide, 3.5 to 4.5 times as long as broad, some late season exceptions are shorter, base attenuate (angle 40 to 50°); laminal margins entire to distantly subserrulate, minutely ciliate; pubescence about 0.1 mm long; sepals linear, acute, auricles with or without pubescence; corolla yellow, tinted brown outside, lateral petals bearded within , lower and lateral petals marked with brown lines within, lower petal 10 to 13 mm long including short (1 mm) spur; stigma short tubular, style capitate, head retrosely bearded at sides; ovary glabrous or sparsely, minutely puberulent; cleistogamous flowers minute, sepals 5 mm long, common throughout growing season; fruit a valvular capsule producing about 10 seeds per capsule; seeds brown, 3 mm long and 1.5 mm wide, weighing 3 mg each; caruncle distally flattened, 1.1 mm long, extending beyond and completely covering the funiculus; pollen 35.2 um in diameter; 2N = 24.

V. nuttallii is widespread east of the Rocky Mountains in the southern regions of Alberta, Saskatchewan and Manitoba, Canada, and in the mid-western United States of Montana, North Dakota, South Dakota, Wyoming, Colorado, Nebraska, and Kansas. It occurs at elevations of 700 to 2400 meters above sea level (Figure 14).

Leaf tissue of specimens from this species was commonly found to contain apigenin, kaempferol, and quercetin flavonoid derivatives.

The specimen designated as the type (Figure 15), "on the sandy plains of the Missouri", appears to be Nuttall's type collection. The plate published in Hooker's *Flora* (1830) has been refered to as the standard drawing of this species (Baker, 1957).

V. nuttallii has been found in association with *Phlox hoodii, Lithospermum incisum, Opuntia polyacantha, Bouteloua gracilis, Zygadenus elegans, Artemisia frigida, Allium textile, Rhus aromatica, Sisyrinchium montanum, Cornus alba,* and, *Rosa acicularis* . All of these species are indicative of the xeric conditions to which *V. nuttallii* appears to be adapted. Field observations indicated that, relative to the other members of the Nuttallianae, *V. nuttallii* occurs in the driest conditions.

Viola nuttallii has been shown to possess a chromosome number of 2N = 24 and shows no evidence of hybridization anywhere within its range. A barrier to gene exchange can reliably be assumed to exist. This taxon is certainly a "good" species

Figure 14. Distribution of *Viola nuttallii* Pursh.

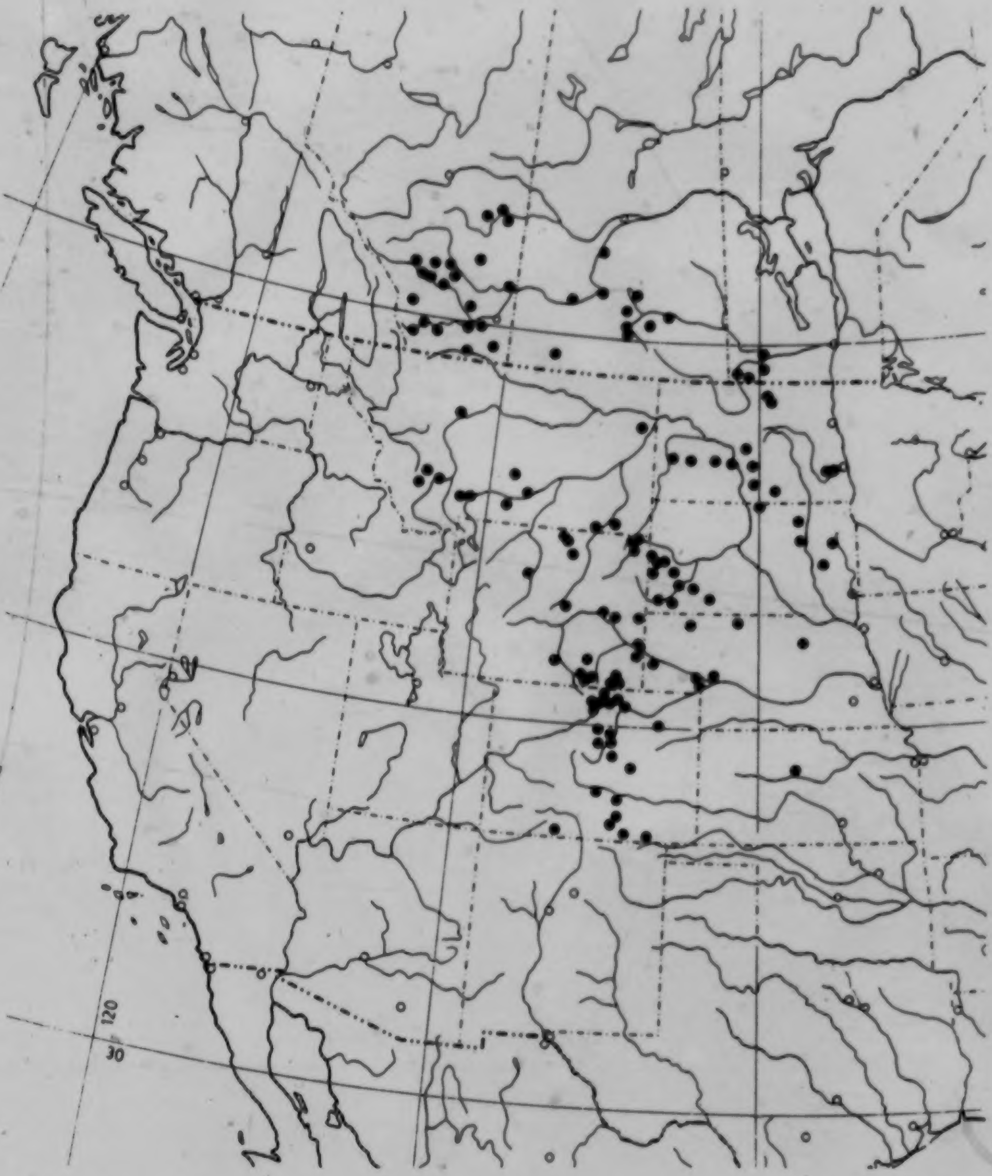
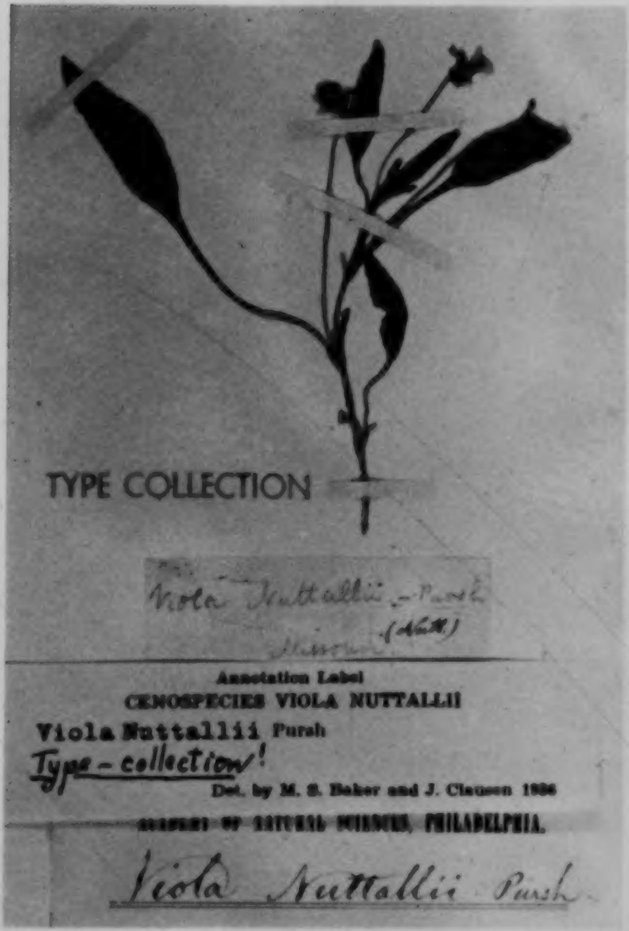


Figure 15. Holotype of *Viola nuttallii* Pursh.



TYPE COLLECTION

Viola Nuttallii — Pursh
(Nutt.)
Missouri

Annotation Label
CENOSPECIES VIOLA NUTTALLII
Viola Nuttallii Pursh
Type-collection!
Det. by M. S. Baker and J. Clausen 1936

ACADEMY OF NATURAL SCIENCES, PHILADELPHIA.

Viola Nuttallii Pursh

according to the definition adopted for this study. Though some variation in leaf shape can be observed, particularily in late season growth, *V. nuttallii* can be reliably distinguished from all other members of the complex by it's leaf shape, short puberulence, and large, brown seeds.

Viola vallicola A. Nels. Bull. Torrey Bot. Club 26: 122-134. 1899.

Type: "Collected in several places in the state, the following numbers well representing it: 43, 4340, 4345, 4525." *A. Nelson 4340*, Pine Ridge, Wyoming. June 7, 1898. RM! (holotype).

Viola glareosa Dougl. MSS, never published.

Viola physolodes Greene. Pl. Baker. 3: 12. 1901.

Type: "In thickets along the Cimarrom River, 7 June, 1901." *C. F. Baker 67*. RM! (isotype).

Crocien vallicola (A. Nels.) Nwd. and Lunnell. Am. Midl. Nat. 4(11): 467-487. 1916.

Viola subsagittifolia Suks. Werdenda 1: 25-26. 1927.

Type: *W. Suksdorf 8530*, 10 km southeast of Spangle, Spokane County, [Washington,] 26 April and 15 May, 1916. WS! (holotype); PH!, UC!, MO! (isotypes).

Viola nuttallii Pursh variety *vallicola* (A. Nels.) St. John. *Fl. S.E. Washington and adjacent Idaho*. 1937.

Viola Russellii Boivin. Can. Field Nat. 65(1): 22. 1951.

Type: "Saskatchewan: R. C. Russell and B. J. Sallons, Duval, Last Mountain, small draw on hillside. May 13, 1940. DAO! (holotype).

Viola nuttallii Pursh subspecies *vallicola* (A. Nels.) Taylor and MacBryde. Can. J. Bot. 56(2): 190. 1978.

Perennial from relatively shallow-seated rootstock; habit decumbent, subcaulescent, stems elongating and erect late in season; leaves ovate to oblong-ovate, 19 to 43 mm long and 9 to 11 mm wide, blades about 2.2 times as long as broad, truncate to subcordate at base (angle 170 to 185°); glabrous to minutely puberulent throughout; margins entire to subentire, ciliate; chasmogamous flower lower petal 9 to 14 mm long, sepals glabrous or puberulent on auricles only; cleistogamous flower sepals 4.5 mm long; ovary or expanding capsule glabrous or sparsely, minutely puberulent; seeds 2.2 mm long and 1.2 mm broad, weighing about 1.6 mg each; caruncle distally flattened, 0.8 mm long, extending beyond the funiculus and covering it, extending along up to 40% the length of the seed; seed coat tan with dark brown edges around caruncle; pollen 31.4 um in diameter; 2N = 12.

Viola vallicola has the widest distribution of any taxon within the Nuttallianae, ranging from south-central British Columbia to southwestern Manitoba and the Dakotas, south to Nevada, Utah, Colorado, and Arkansas (Figure 16). It occurs at elevations of 400 to 2800 m. The distribution appears to be continuous across mountain ranges, particularily through the intermountain region of Nevada and Utah. Flowering time was common throughout May though they were not uncommonly observed anytime from late March to early July.

V. subsagittifolia Suksdorf from Washington certainly represents *V. vallicola* and must be included here. The new combinations published by St. John (1937) and Taylor and MacBryde (1978) were meant to refer to the taxon located in Washington and British Columbia, respectively. As no specimens were available from which to obtain flavonoid profiles, it was considered more logical to refer these combinations to the specific level. The C. F. Baker specimen from eastern Colorado also must be the eastern chemical form. Though no chemical extractions could be made, it is possible that this collection may be closer to the intermountain intermediate than to the eastern form.

Crocien vallicola Nwd. and Lll. was recombined as a result of a desire to subdivide the genus *Viola* into several genera based on plant caulescence and type of cleistogamous flowers, if present (Niewland and Kaczmarel, 1914). This division into seven new genera and three subgenera was never widely adopted. The study was based on the examination of very few species, and in fact did not include any members of the *nuttallii* complex. The section and subsection notation as used in this study was much more widely accepted and used since it was thought to reflect *Viola* as a natural group.

Boivin's description of *V. russellii* (1951) represents a species whose attributes match those of *vallicola* and whose type was located in the range of the eastern subspecies, *vallicola*. The publication gives no explanation for segregating *russellii*

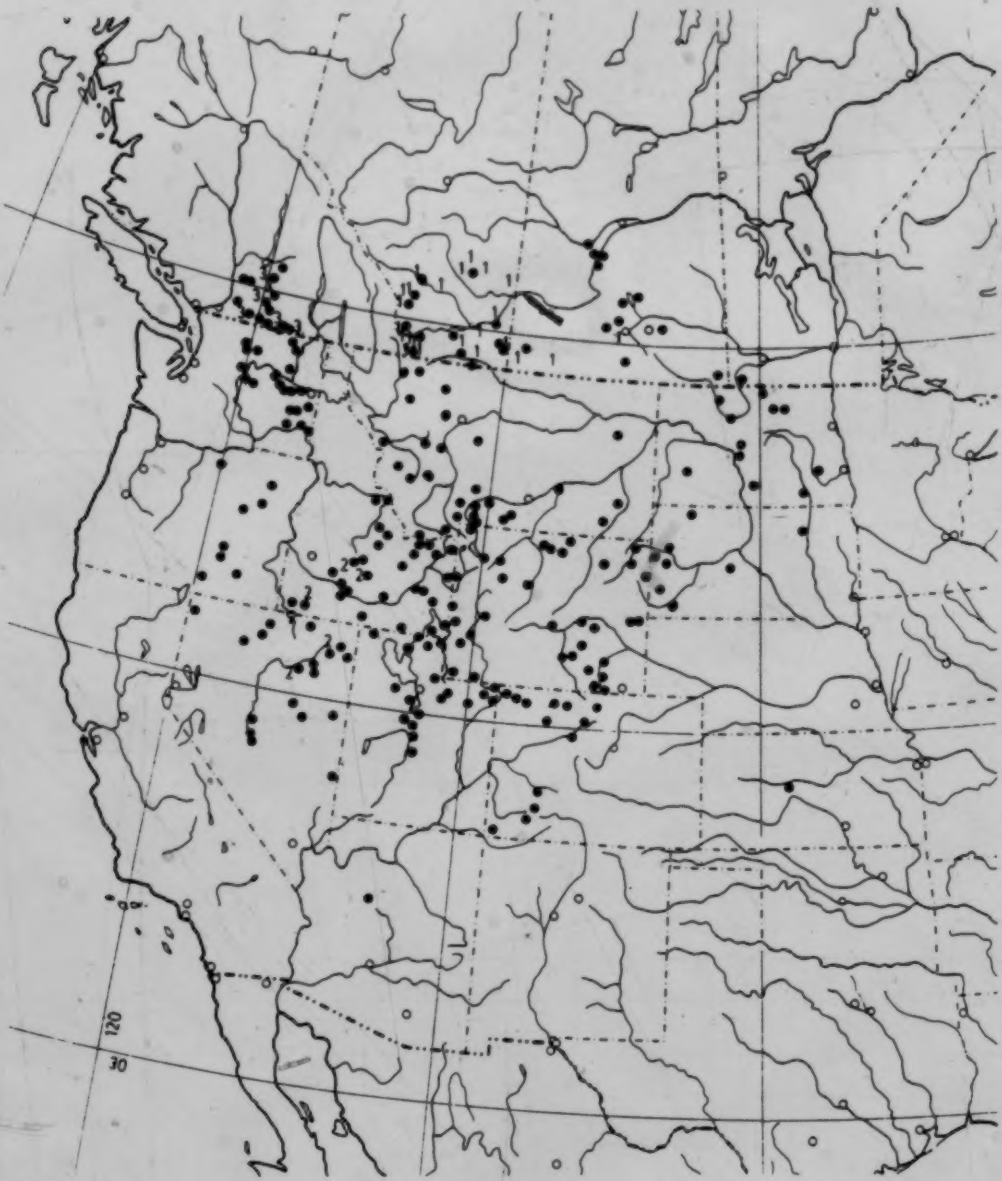


Figure 16. Distribution of *Viola vallicola* Nels.

Black dots represent specimens of *V. vallicola*, 1= subspecies *vallicola*, 2= flavonoid intermediates, and 3= subspecies *major*.

and *vallicola* though in a reply to an inquiry from M. Bowerman (1952, see DAO 288885) Boivin makes it apparent that no type specimens were examined and that he found it "difficult . . . to state the differences between these two species." Listed paratypes were from British Columbia, Alberta, and Montana, and the type was from Saskatchewan. This taxon was considered as synonymous with *V. vallicola*.

V. vallicola does display some variability in the size of leaves, degree of basal truncation, stem elongation, and chasmogamous flower size. Despite these minor variations, specimens of this species can easily be distinguished from *nuttallii* by leaf shape, and from other Nuttallianae by their near glabrous leaves.

Two chemotypes were observed within this species which likely represent two extremes in a range of variability.

Key to the subspecies of *Viola vallicola* A. Nels.

1. a. Leaf flavonoids primarily kaempferol derivatives; found west of the continental divide . . . . subspecies *major*

1. b. Leaf flavonoids all apigenin derivatives; found east of the Rocky Mountains . . . . .subspecies *vallicola*

Viola vallicola A. Nels. subspecies **major** (Hook.) Fabijan comb. nov.

Viola nuttallii variety *major* Hook. Fl. Bor. Am. 70. 1830.

Type: "Abundant under to shade of pines on the dry sandy plains of the Columbia." KEW! (holotype), UC! (photo of holotype).

Viola nuttallii major (Hook.) Piper. Contr. U. S. Nat. Herb. 11: 393. 1906.

Viola praemorsa variety *major* (Hook.) Peck. Madrono 6: 135. 1941.

Viola praemorsa Dougl. subspecies *major* (Hook.) Baker. Madrono 10:128. 1949.

Leaf flavonoids primarily kaempferol derivatives; occurring west of the continental divide.

Subspecies *major* occurs west of the Rocky Mountains in British Columbia and Oregon (number 3 in Figure 16).

William Hooker (1830) described *major* as having wider leaves and larger flowers than *V. nuttallii*. The only specimen referred to was a Douglas collection which he had described in manuscript as a new species, *V. glareosa*. This specimen (H 1526/82) was from Herbariorum Hookeriana, was designated as the type at KEW (Figure 17). The label reads "*Viola glareosa* abundant under shade of solitary pines on dry sandy soil of Columbia." There can be little doubt of its application to the epithet "*major*", and thus, its connection to the western flavonoid form of *V. vallicola*.

Viola vallicola A. Nels. subspecies **vallicola**

Viola vallicola A. Nelson. Bull. Torrey Bot. Club 26: 122-134. 1899.

Type: "Collected in several places in the state, the following numbers well representing it: 43, 4340, 4345, 4525." *A. Nelson 4340*, Pine Ridge, Wyoming. June 7, 1898. RM! (holotype).

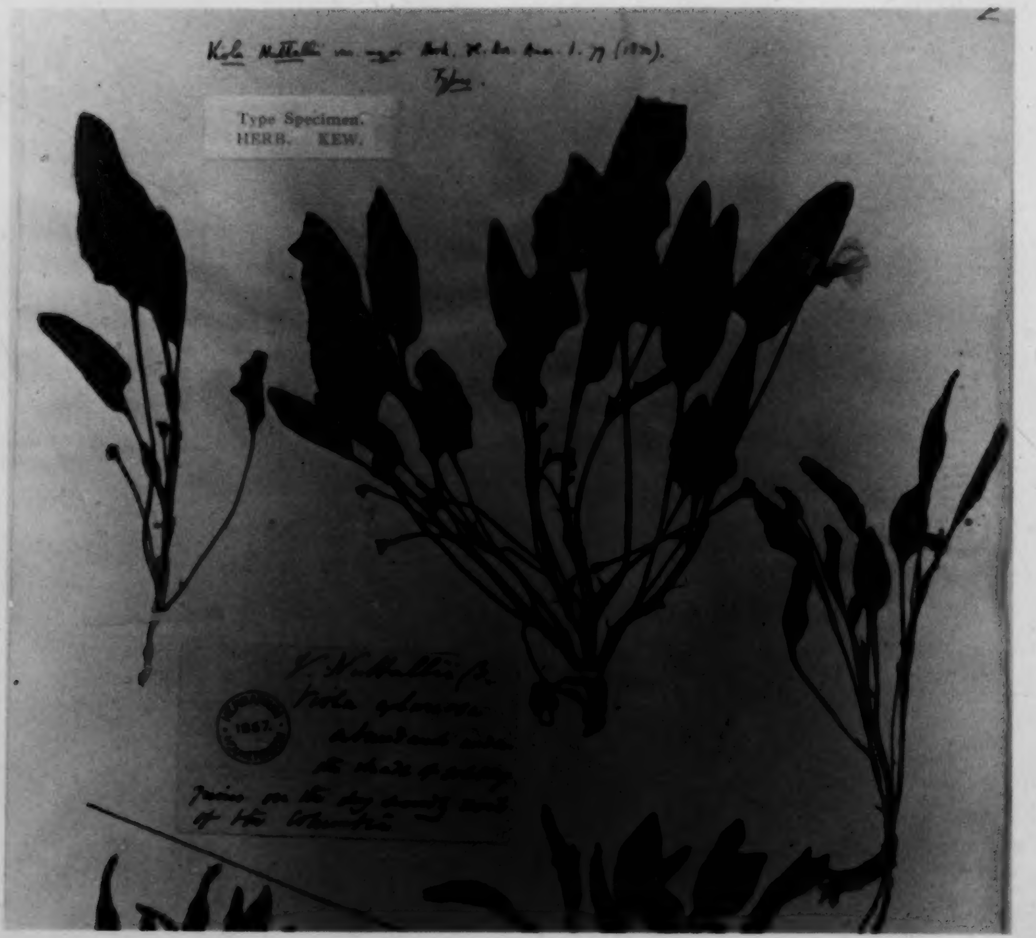
Leaf flavonoids all only apigenin flavones; occurs east of the continental divide in Alberta and Saskatchewan.

Though there was a tendency to shorter, wider cauline leaves, the range of variation was too great to be significant. The only reliable means by which to make identifications being geographical, it was thought that distinction at subspecies level was acceptable between *major* and *vallicola*.

The specimens cited by Nelson (1899) were all collected in Wyoming. Though number 43 was the first number refered to in publication, number 4340 was designated as the type specimen in the Rocky Mountain Herbarium. Pine Ridge is in the Black Hills in Crook County, northeastern Wyoming and logically may be refered to the eastern flavonoid form.

The two chemical forms, *major* and *vallicola*, could not be reliably distinguished on the basis of any morphological character examined in this study. Although there was a tendency for *major* specimens to display slightly smaller leaf length-to-width ratios, and

Figure 17. Holotype of *Viola vallicola* A. Nels. subspecies *major* (Hook.) Fabijan.


Viola Nuttallii var. major Hook. Fl. Bor. Amer. 1. 77 (1830).
Type.
Type Specimen.
HERB. KEW.
1867.
V. Nuttallii β.
Viola glomerosa
abundant under
the shade of ...
pines on the dry sandy ...
of the Columbia

subentire, ciliate leaf margins as compared to *vallicola*. As noted in the morphological results, two specimens of *major* showed longer or darker seeds. None of these attributes were consistent or significant.

The only reliable means of identifying the subspecies appeared to be flavonoid chemistry, with apigenins commonly found in *vallicola* and kaempferols in *major*. These two taxa are not geographically isolated except in the northern extreme of the range. Genetic isolation cannot be assumed between the subspecies which likely intermingle, particularily in the intermountain regions of Idaho and Nevada. Examination of the leaf flavonoids from specimens in that area (OTUs 77, 78, 79, 80, 81: B9354, D1754, D1732, D1753, and D1736, respectively) contained apigenin and kaempferol derivatives.

Viola tomentosa Baker and Clausen. Leafl. West. Bot. 5: 141-147. 1949.

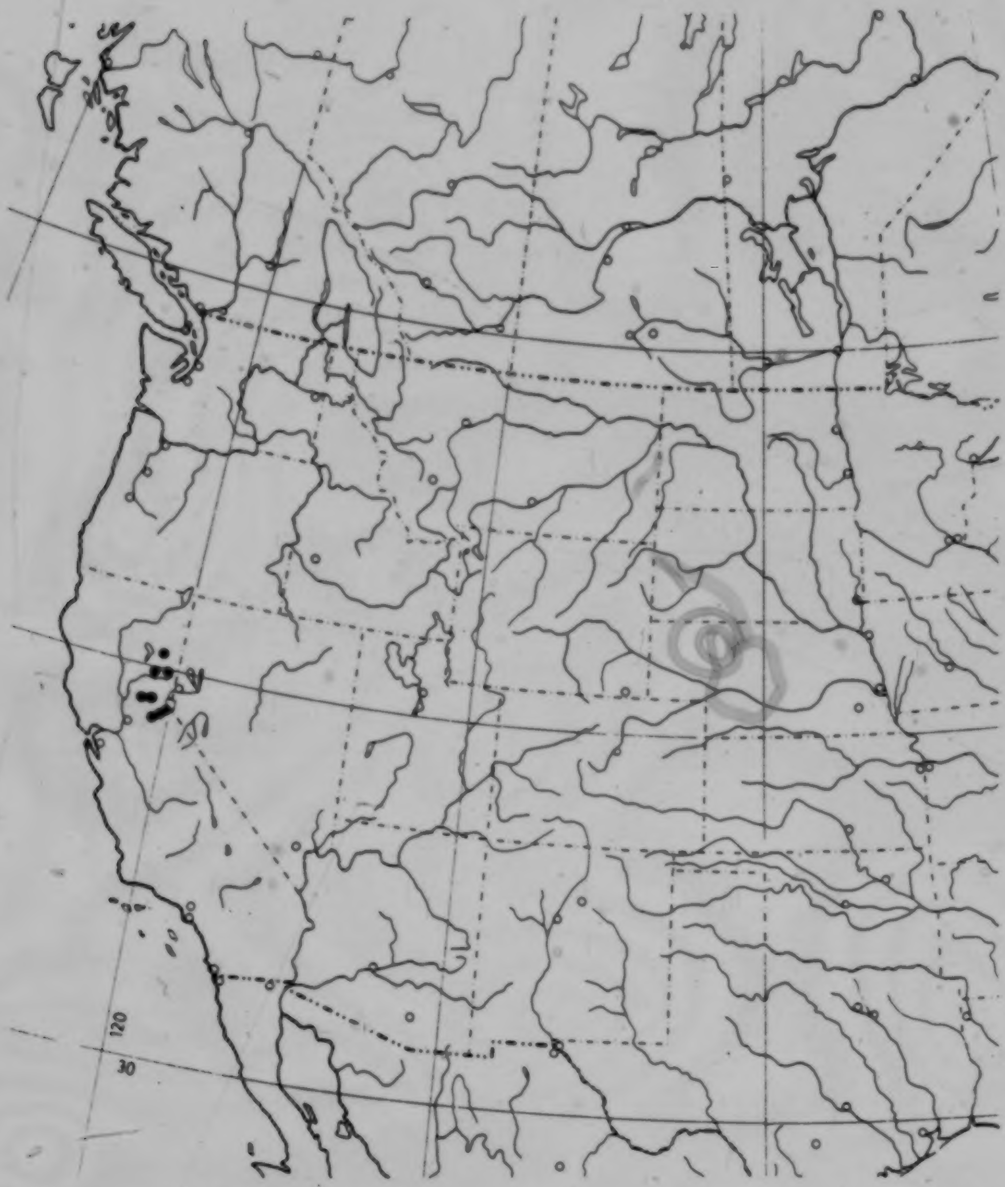
Type: *M. S. Baker 8722A*, "July 9, 1937 (early growth), . . . collected along California highway 20, about 13 miles westerly from Cisco, Nevada Co., California, at about 5000 ft. elevation in forests of *Pinus ponderosa, Quercus Kelloggii*, and *Libocedrus decurrens*." CAS (holotype), UC!, OSU, MO, NY, US, and G (isotypes).

Plant densely woolly tomentose throughout; leaves 15 to 50 mm long, 9 to 25 mm wide, ovate to elliptic, margins entire; chasmogamous flowers small, lower petal about 9 mm long; cleistogamous flowers unknown; 2N = 12.

In terms of leaf flavonoids, *tomentosa* contained kaempferol, apigenin, and quercetin as had *nuttallii*. This was intermediate between *major*, and *praemorsa* and *bakeri*. *V. tomentosa* appears to be rare and restricted to the Sierra Nevada of California at elevations of 1500 to 2000 m (Figure 18). It has been found in dry gravelly soil of open coniferous forests. *V. tomentosa* has an extended flowering season from June to August.

V. tomentosa was without a doubt distinctive both in terms of its morphology and its diploid chromosome complement.

Figure 18. Distribution of *Viola tomentosa* Baker & Clausen.



Viola bakeri Greene. Pittonia 3: 307. 1898.

Type: Bear Valley mountains, Siskiyou County, California. June, 1896. UC (holotype), DS! (isotype).

Viola bakeri Greene subspecies *grandis* Baker. Madrono 10: 117. 1949d. (Nom. Nud.)

Viola bakeri Greene subspecies *shastensis* Baker. Madrono 15: 203-204. 1960.

Type: *Baker 13045.* "Postpile Camp, altitude 6000 ft., western Tehama County, California." July 1, 1955. UC! (holotype).

Viola nuttallii A. Nelson variety *bakeri* (Greene) Hitchcock. *Vascular plants of the Pacific Northwest* 3: 447. 1961.

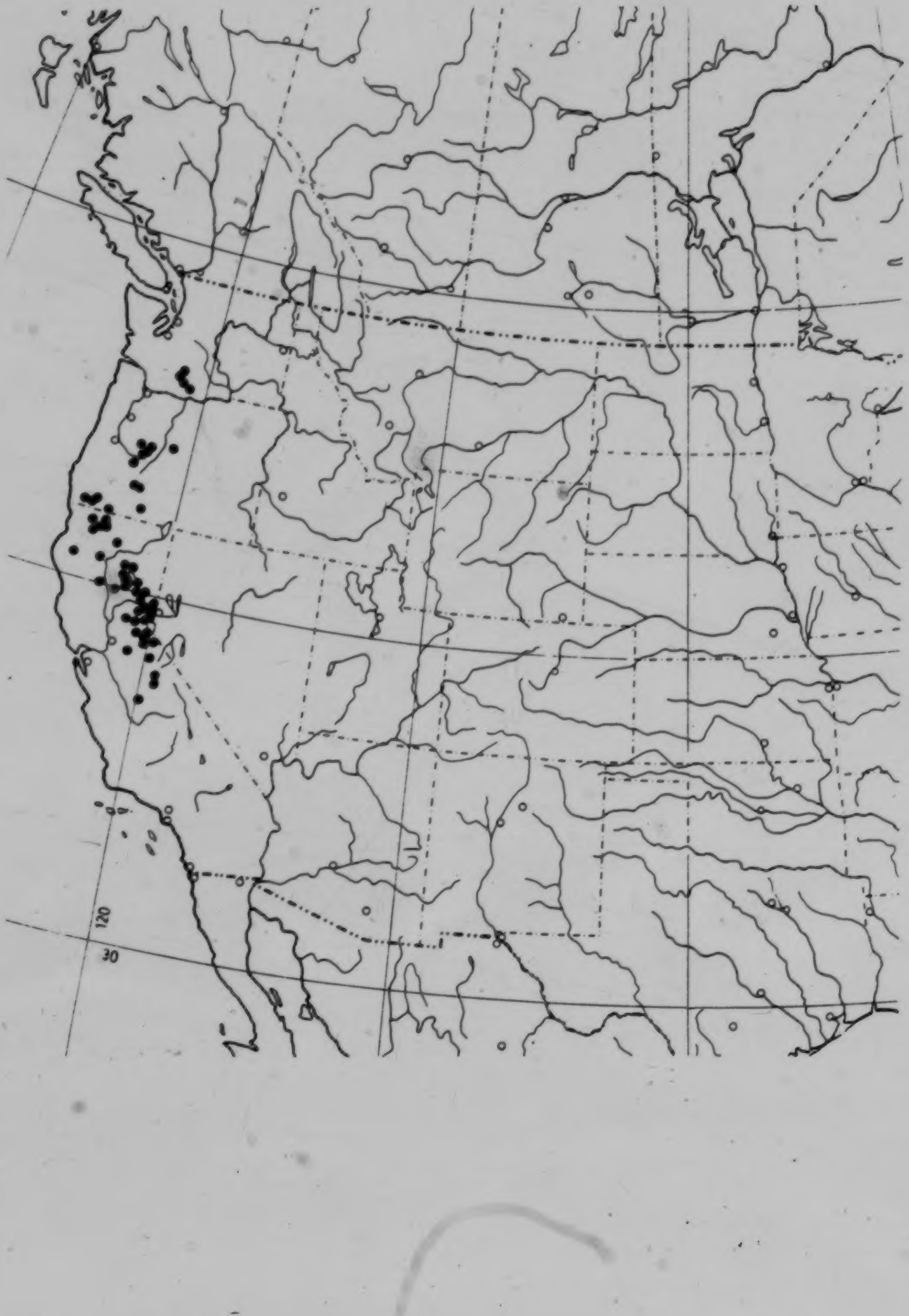
Viola bakeri Greene subspecies *puberulenta* Baker (mss., not published).

Subcaulecent early in the season and displaying elongated stems in later growth; radical and cauline leaves 20 to 40 mm long and 10 to 15 mm wide, ovate-lanceolate to ovate or elliptical, 2.1 to 2.7 times as long as wide, bases cuneate (55 to 80°), margins always entire and ciliate; more or less pubescent throughout, hairs 0.3 to 0.4 mm long, occasionally hirsute; lower chasmogamous flower petal 10 to 15 mm long; style capitate and retrosely bearded on sides as in all Nuttallianae; cleistogamous flowers 3.5 to 8.0 mm long; seeds dark red-brown, 1.8 mm wide and 3.1 mm long, 1.7 times longer than wide, weight 4.2 mg; caruncle 29% the length of the seed but not extending beyond the tip of the seed and covering the funiculus; pollen 44 um in diameter; 2N = 48.

Viola bakeri was distributed from the Cascades of southern Washington to the Sierra Nevada of California (Figure 19). It occurs at elevations from 900 to 3800 m, most commonly above 1700 m. Flowering time peaked from mid June to mid July, but sometimes occurred as early as May and as late as August.

Bakeri may be easily distinguished from *tomentosa* by the shorted length and lower density of its pubescence, by its chromosome number and by its more diverse flavonoid profile. Leaves of *bakeri* contained luteolin derivatives, as well as apigenin, kaempferol and quercetin derivatives.

Figure 19. Distribution of *Viola bakeri* Greene.



Viola bakeri may be distinguished from *nuttallii* primarily by its entire leaf margins and by its distribution east of the Rocky Mountains. The true distinction would be chromosome number, *bakeri* is an octoploid and *nuttallii* is a tetraploid. The characters of ovary pubescence and margin ciliation were not always consistent within the taxa and were, therefore, not applicable in distinguishing them (Table 8).

Baker (1949c) mentioned two subspecies *typica* and *grandis* without descriptions or explanations. The invalid subspecies *typica* should properly have been refered to as *bakeri*, priority being given to the oldest published applicable epithet. Subspecies *grandis* was not validly published in receiving no latin description or designated type specimen. Baker (1960) maintained *grandis* as "nomen nudum". He deferred properly describing it due to a lack of continuity in its range.

The subspecies *shastensis* (Baker, 1960) was described as differing from *bakeri* by the presence of short appressed hairs on the sepals and capsules. These features (Table 8), were not consistently reliable in distinguishing between taxa. Creating a new subspecies based on inconsistent character states could not be justified by this study, and the name was considered synonymous with *bakeri*.

Viola praemorsa Dougl. ex Lindl. Edward's Bot. Reg. 15: pl. 1254. 1829.

For synonymy and typification see subspecies headings.

An herbaceous perennial from a short, occasionally praemorse rootstock; appearing acaulescent but stem elongating during the growing season; 6 to 20 cm high; glabrous or conspicuously hirsute throughout; radical leaves various but mostly broadly ovate to obovate; cauline leaves ovate to oblong-lanceolate, base cuneate to truncate or subcordate; margins sub-entire to serrate; corolla of chasmogamous flowers yellow, lower petal up to 20 mm long including the spur; style 2.5 mm long, capitate head retrosely bearded on sides; capsule glabrous to puberulent; cleistogamous flower sepals 3.5 to 8.3 mm long; seeds of various colours from medium to dark brown; mostly 1.6 times longer than wide; caruncle short, globose, not extending beyond the seed apex or covering the funiculus; pollen diameter 41 to 45 um; 2N = 36, 48.

V. praemorsa occurs in the western half of the Nuttallianae range from southwestern Alberta and the Intermountain regions of Utah west through the Great Basin to Vancouver Island and central California.

Viola praemorsa is broadly defined here, in the tradition of Baker (1957), based on morphological variations in leaf shape, pubescence, and seed morphology. *V. praemorsa* specimens can be easily recognized by their hirsute pubescence, if present, larger leaf size, and the distinct caruncle form of the seeds. A wide array of structural variation exists in this species throughout its range. The three subspecies recognized in this study exhibited gradual intergradation whose intermediates are difficult to identify and classify. The influences of physical and environmental factors, along with hybridization and introgressive influences contribute to the multifarious nature of the structural forms observed. These subspecies represent variants defined primarily on extremes in leaf form, whose character states tend to overlap. The relationships of these taxa are discussed in more detail later.

Key to the subspecies of *Viola praemorsa*

1. a. Cauline leaves mostly short, 23 to 40 mm long; ovate to broadly ovate, length to width ratio of 1.6; blade base approaching truncate (106 to 172°), margins serrate; seeds dark brown, widely ovate, length to width ratio 1.4, 5.8 mg each, caruncle relatively short, 0.7 mm long; 2N = 36. . . . . subspecies *praemorsa*

1. b. Cauline leaves mostly longer, 33 to 80 mm; ovate to elliptical, length to width ratio of 1.8 to 3.2; basal angle 63 to 112°, margins subserrate to serrate; seeds medium brown, ovate, 1.5 to 1.7 times longer than wide, 3.9 to 5.1 mg; caruncle 0.8 to 1.0 mm long;2N = 36, 48.

  2. a. Cauline leaves 33 to 62 mm long, 15 to 23 mm wide, ovate to broadly ovate; 2N = 36, 48 . . . . .subspecies *linguaefolia*

  2. b. Cauline leaves large, 60 to 80 mm long, 23 to 31 mm wide, elliptic; 2N = 36, 48 . . . . subspecies *flavovirens*

Viola praemorsa Dougl. ex Lindl. subspecies **praemorsa**

Viola praemorsa Dougl. ex Lindl. Edward's Botanical Register 15: plate 1254. 1829.

Type: "According to Mr. Douglas, in dry upland soils, under the shade of solitary Pine trees on the banks of the Columbia, and the plains above the river Aguilar, in California. Our drawing was made in the Garden of the Horticultural Society in 1828." CGE (holotype), DS! (photograph of holotype).

Lectotype: *M. S. Baker 9634,* South Tacoma, Washington. UC! (lectotype); WS!, RM!, MO!, DAO! (isolectotypes). Lectotypification redundant.

Viola nuttallii variety *praemorsa* (Dougl. ex Lindl.) S. Wats. *Botany, Clarence King Expedition; U.S. Geological Exploration of the Fortieth Parallel* 5: 35. 1871.

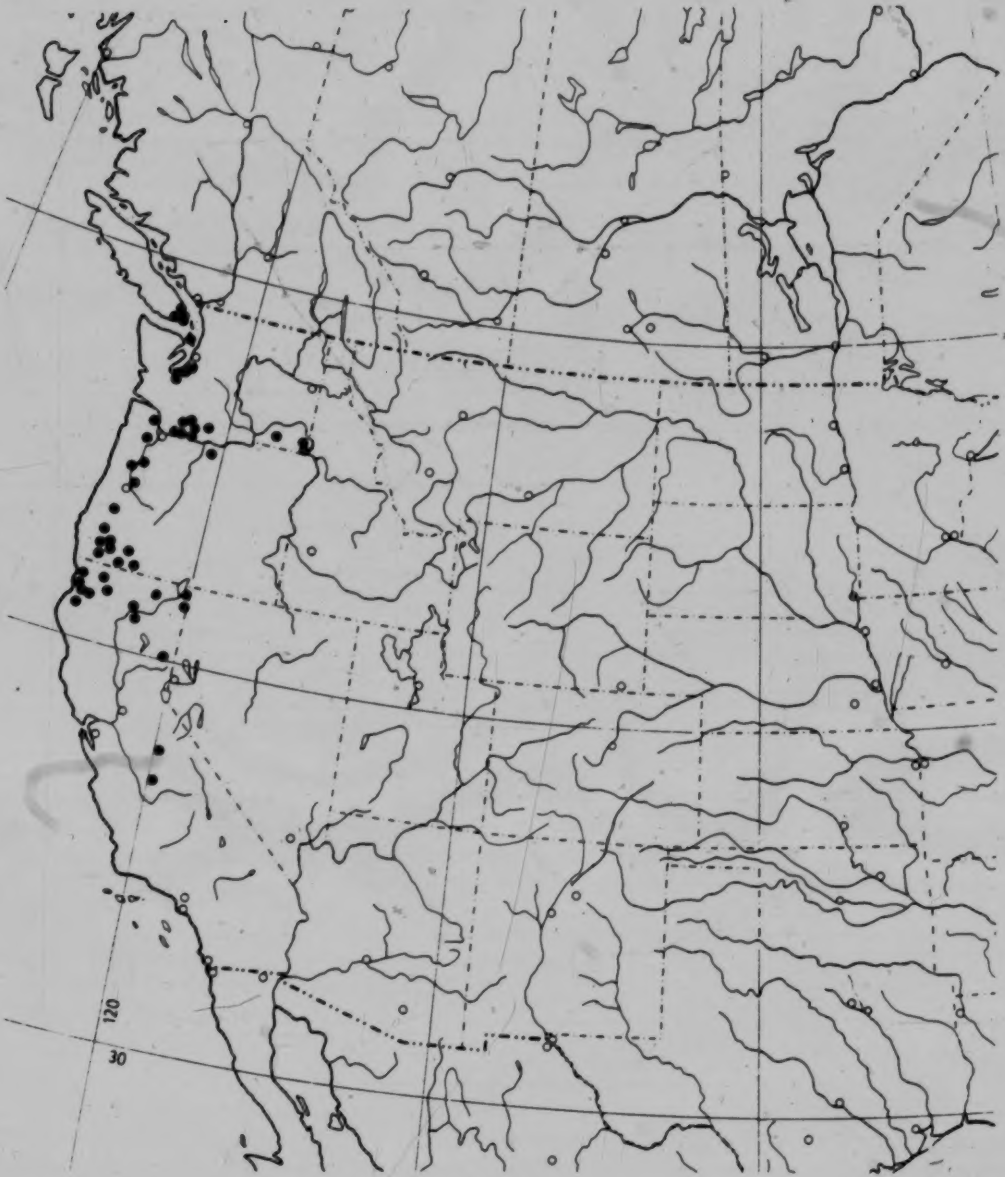
Viola nuttallii praemorsa (Dougl. ex Lindl.) Piper. Contr. U.S. Nat. Herb. 11: 393. 1906.

A herbaceous perennial, with short leaves, 20 to 40 mm long, length to width ratio of 1.6; leaf bases approaching truncate; pubescence densely hirsute at least on petioles and young leaves; seeds widely ovate, length to width ratio only 1.4, and weighing 5.8 mg, caruncle relatively short, 0.7 mm long, seed coat dark brown; 2N = 36.

This taxon occurs from the Saanich Peninsula of Vancouver Island south along the Coastal Ranges to northern California (Figure 20). Invaginations inland were evident along the Columbia River in Washington and toward the Sierra Nevada in California. Subspecies *praemorsa* was commonly found at elevations of 100 to 2500 m, though a few inland collections were found at elevations up to 7000 m. Flowering times were generally middle of April to early May but have been observed from March to June.

This taxon was first collected by D. Douglas near the mouth of the Columbia River. The published drawing was made from transplanted specimens at the Botanic Garden, but the original specimens appeared to have been lost. Most workers accepted the drawing in lieu of a holotype (Brainerd, 1921). From a photograph (DS 558332), it appears that a collection purchased from Lindley's herbarium had found its way to Cambridge and may well be the original type specimen. The label reads: "North West America, Douglas."

Figure 20. Distribution of *Viola praemorsa* Dougl. ex Lindl. subspecies *praemorsa*.



"Cambridge Botanical Museum. Herb. J. Lindley, PhD. Purchased 1866." Baker (1949c) lectotypified this taxon with a collection from Tacoma, Washington which is considered to be redundant.

Viola praemorsa Dougl. ex Lindl. subspecies **linguaefolia** (Nutt. ex T. & G.) Baker. Brittonia 9: 226. 1957.

Viola linguaefolia Nutt. ex T. & G. *A Flora of North America* 1: 141. 1838.
Type: "Kamas prairie, near the sources of the Oregon, Mr. Wyeth." PH! (holotype); GH! (photograph of holotype), WS! (photograph of holotype); BM! (isotype); PH! (photograph of isotype).

Viola erectifolia A. Nels. Botanical Gazette 29: 143. 1900.
Type: *A. Nelson 5481*, Henry's Lake, Idaho, June 22, 1899. RM! (holotype); PH!, MO!, RM!, UC!, US!, and GH! (isotypes); ALTA (topotype, DF 622).

Viola gomphopetala Greene. Plantae Bakerianae 3: 11. 1901.
Type: *C. F. Baker 225*, on open hillsides of Grand Mesa [Colorado], June 23, 1902. RM! (holotype); RM!, WS!, UC!, GH! (isotypes).

Viola nuttallii linguaefolia (Nutt. ex T. & G.) Piper. Flora of Southeast Washington. 1914.

Viola nuttallii Pursh variety *lingulaefolia* (Nutt. ex T. & G.) Jeps. *A Man. Fl. Plants of Calif.*, 645. 1925.

Viola xylorrhiza Suks. Werdenda 1: 25. 1927.
Type: *W. Suksdorf 10200*, east of Husum, Klickitat County, Washington, 10 May, 30 June, 1919 and 1 June, 1920. WS! (holotype); UC!, MO!, CAS!, and DAO! (isotypes); ALTA (topotype, DF 397).

Viola praemorsa variety *oregana* (Baker and Clausen) Peck. *Man. High. Plants of Oregon*, 486. 1941.

Viola praemorsa variety *linguaefolia* (Nutt. ex T. & G.) Peck. Madroño 6: 135. 1941.

Viola praemorsa Dougl. ex Lindl. subspecies *arida* Baker. Brittonia 9: 227-228. 1957.
Type: *M. S. Baker 11462* in fruit, outskirts Klamath Falls, Oregon. UC! (holotype); RM!, DS!, MO!, and DAO! (isotypes).

Paratype: M. S. Baker 12086 in flower, from same colony. UC! (holotype); MO!, RM!, DAO! (isotypes); GH! (photograph of holotype).

Viola praemorsa Dougl. ex Lindl. subspecies *oregona* Baker. Brittonia 9: 228-229. 1957.

Type: *M. S. Baker 8862*. "The type locality is about half mile west of Klamath River Bridge on the Klamath Falls to Medford hwy c. 20 miles from Klamath Falls." DS! (holotype); RM!, MO!, and DAO! (isotypes); GH! (photograph of holotype). ALTA (topotype, DF 443).

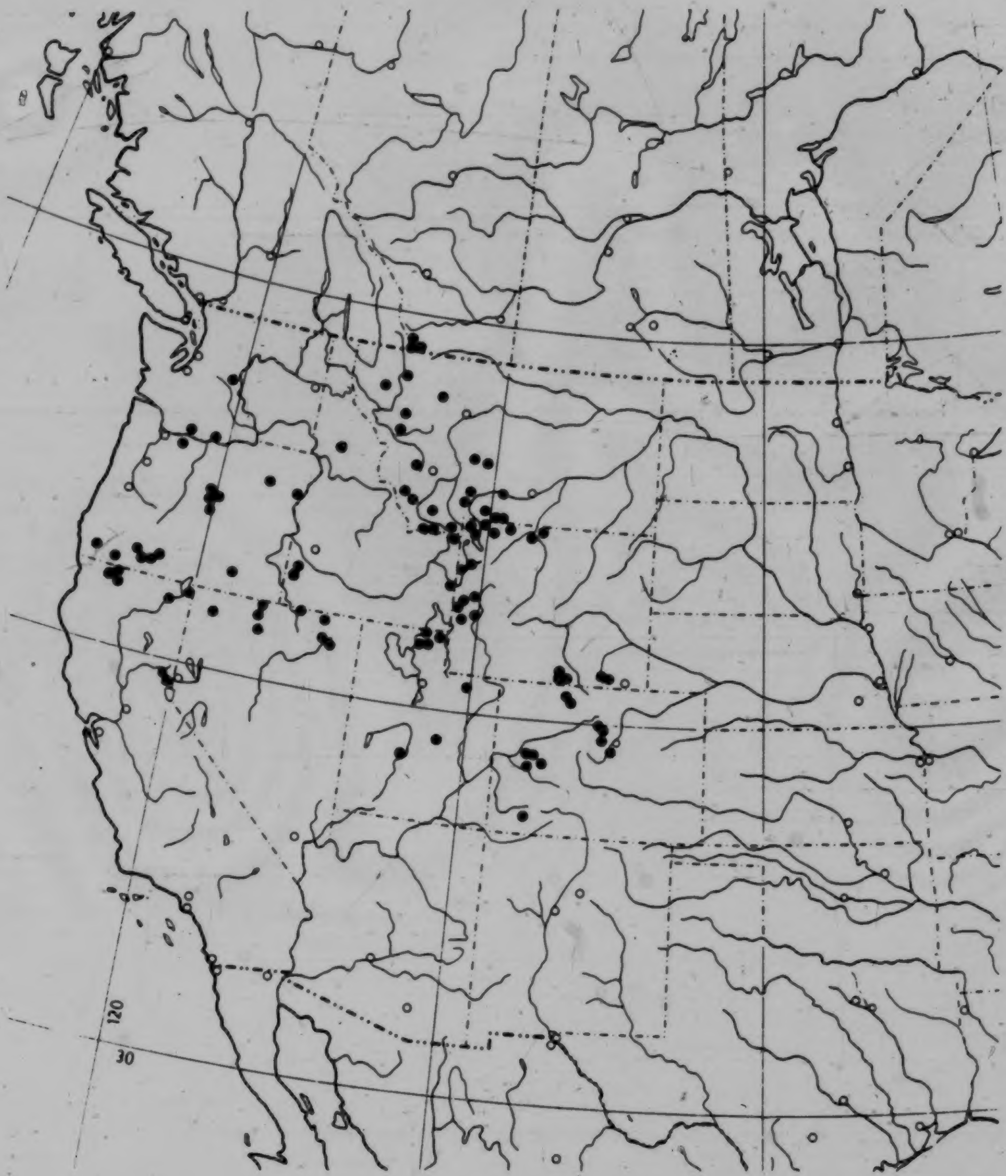
Leaves tongue-shaped, 33 to 62 mm long and 15 to 23 mm wide, ovate to narrowly ovate, blades cuneate (63 to 112°), margins subserrulate to serrulate; seeds brown to red-brown, 1.5 times longer than wide, weighing up to 5.1 mg with caruncles less than 1 mm in length; pollen large, 37 to 45 um in diameter; 2N = 36, 48.

Occurs from the Rocky Mountains of southwestern Alberta through Wyoming, Colorada and Utah, through the Intermountain region into the Great Basin ranges in northern Nevada and California, Oregon and Washington (Figure 21) at elevations of 1300 to 3500 meters above sea level. The peak flowering times were from mid-June to early August, but some flowers appeared as early as the first week of May. This distribution range overlapped significantly with *bakeri* in central Washington, Oregon, and California; with subspecies *praemorsa* along the Columbia River and in the Sierra Nevada; with subspecies *flavovirens* in Idaho, Washington and Wyoming; and, with *tomentosa* in the Sierra Nevada. The many morphological variants which were observed in this study may be symptomatic of these sympatric distributions which lead to the possibilities of hybridization and/or introgression.

The confusion in this taxon arises from the range of variation which exists in leaf shape and pubescence, and the presence of two different chromosome complements. The confusion in the relationships of the subspecies of *praemorsa* can be illustrated by the number of combinations which have been published by various authors.

A specimen at PH was annotated as the type specimen of *Viola linguaefolia*. It's label read "*V. nuttallii / linguaefolia* / HK... *Hookeri. Kamas Prairie near the source of the Columbia (Nutt. from Wyeth)." This label matches the information given in

Figure 21. Distribution of *Viola praemorsa* Dougl. ex Lindl. subspecies *linguaefolia* (Nutt. ex T. &G.) Baker.



the publication (Nutt. ex T. & G.). The handwritting on this specimen also matches that of a specimen located in the British Museum (photographs!) dated June 20. The BM specimen was surely an isotype of the original collection on the Columbia, formerly known as the Oregon River. Nuttall was in Philadelphia from 1836 to 1841 (Brainerd, 1921), during the time when Torrey and Gray published their *Flora* (1838), and it seems most likely that the PH specimen is the holotype.

As shown in the numerical analysis *erectifolia* A. Nels. and *gomphopetala* Greene were both consistently associated with *linguaefolia*. Both were collected in mountainous regions within the range of *linguaefolia*.

Another source of confusion arises from the discovered misapplication of the epithet "*major*" to this taxon. Piper (1906) listed four specimens as representative of *V. nuttallii major*. Three of these specimens were examined and found to be referable here rather than with *major sensu* Hook. in both appearance and location. In listing *major* here, a confusion as to its correct application began which has been perpetuated to this day. It was apparent that Piper did not see the original specimen; if he had seen the specimen there would have been no need to cite Hooker's authority for the synonomy of *glareosa* Dougl. and *major*.

The description and distribution of Jepson's combination (1925) matched perfectly that of *linguaefolia*, though he cited no representative specimens.

It was almost one hundred years after Douglas published *praemorsa* (1829) that workers began to recognize *linguaefolia* as being more closely affiliated with *praemorsa* than with *nuttallii*. Morton Peck (1941) was the first to combine *linguaefolia* and *major* as varieties of *praemorsa*, though no explanations were given. Baker (1949c) listed both of these taxa as subspecies of *praemorsa*. It was not apparent whether type specimens had been examined, but detailed descriptions, distributions, and lists of representative specimens included at the time of publication permited these combinations to be equated here. Baker offered several methods by which to distinguish *major* and *linguaefolia* as he understood them, including leaf and capsule pubescence, leaf width and basal angle, and chromosome complements of 2N = 48 and 36, respectively. As already mentioned, relative puberulence of capsules and density of leaf pubescence have been shown to be inconsistent (Table 8), and therefore, unreliable as distinguishing characters. The extremes of leaf shape used to define these two conceptual subspecies have been shown to represent the ends of a continuum between which no lines could be drawn based on present knowledge.

It is curious to mention at this point that Peck (1941) had published a new combination of *oregana*, apparently a spelling mistake, before the subspecies was actually described (Baker, 1957). Since no explanation was given, one can only assume that he had prior communication with Baker and Clausen before the 1957 publication.

Baker's subspecies *oregona* and *arida* (1957) were shown, in the numerical analysis (Figure 13, Appendix 4), to be morphologically indistinguishable within the continuum of attributes representing subspecies *linguaefolia*. Subspecies *arida* was distinguished because it was octoploid and had puberulent cleistogamous capsules in comparison to the glabrate ones in *major sensu* Baker and *praemorsa*. Subspecies *arida* had been found in a few scattered locations in eastern and southern Oregon and northern California. This range overlaps those of *linguaefolia* and *praemorsa*. I agree with Clausen (1964) who believed that the creation of a new subspecies based on tenuous characters within the range of a taxon from which it could not be reliably distinguished was unjustifiable.

Subspecies *oregona* is restricted to four sites in southern Oregon. It is distinguished by shorter than normal pubescence for the *praemorsa* group, with a leaf shape approaching that of *bakeri* but with serrate margins. The numerical analysis included this taxon morphologically with *linguaefolia* but chemically as intermediate between *linguaefolia* and *bakeri* (Appendix 4). This evidence supports the suggestion of possible hybridization between the two (Baker, 1957) due to their cytological, morphological and flavonoid similarities in conjunction with their overlapping distributions.

The broadly defined subspecies *linguaefolia* was observed to display at least two different flavonoid patterns. In one group, 12 to 20 different flavones and flavonols (apigenin, luteolin, kaempferol, and quercetin derivatives) were present in each specimen extracted. The types of flavonoids and their patterns resembled those found in *bakeri* and *flavovirens*. The second group was found to contain only flavonols, derivatives of quercetin and kaempferol. The profiles of these variants represents a decrease in the diversity of flavonoids to only seven compounds in total. No correlation with morphology, cytology or geographic distribution could be made for these two chemical variants except that all of the reduced profile specimens had a chromosome count of 48.

Viola praemorsa Dougl. ex Lindl. subspecies **flavovirens** (Pollard) Fabijan stat. et comb. nov.

Viola flavovirens Pollard. Bull. Torrey Bot. Club 24: 405. 1897.

Type: *A. A. Heller 3156* "at Lake Waha, Nez Perces Co., Idaho, June 3 and 4, 1896". PH! (holotype); DAO!, MO!, WS!, UC!, and US! (isotypes).

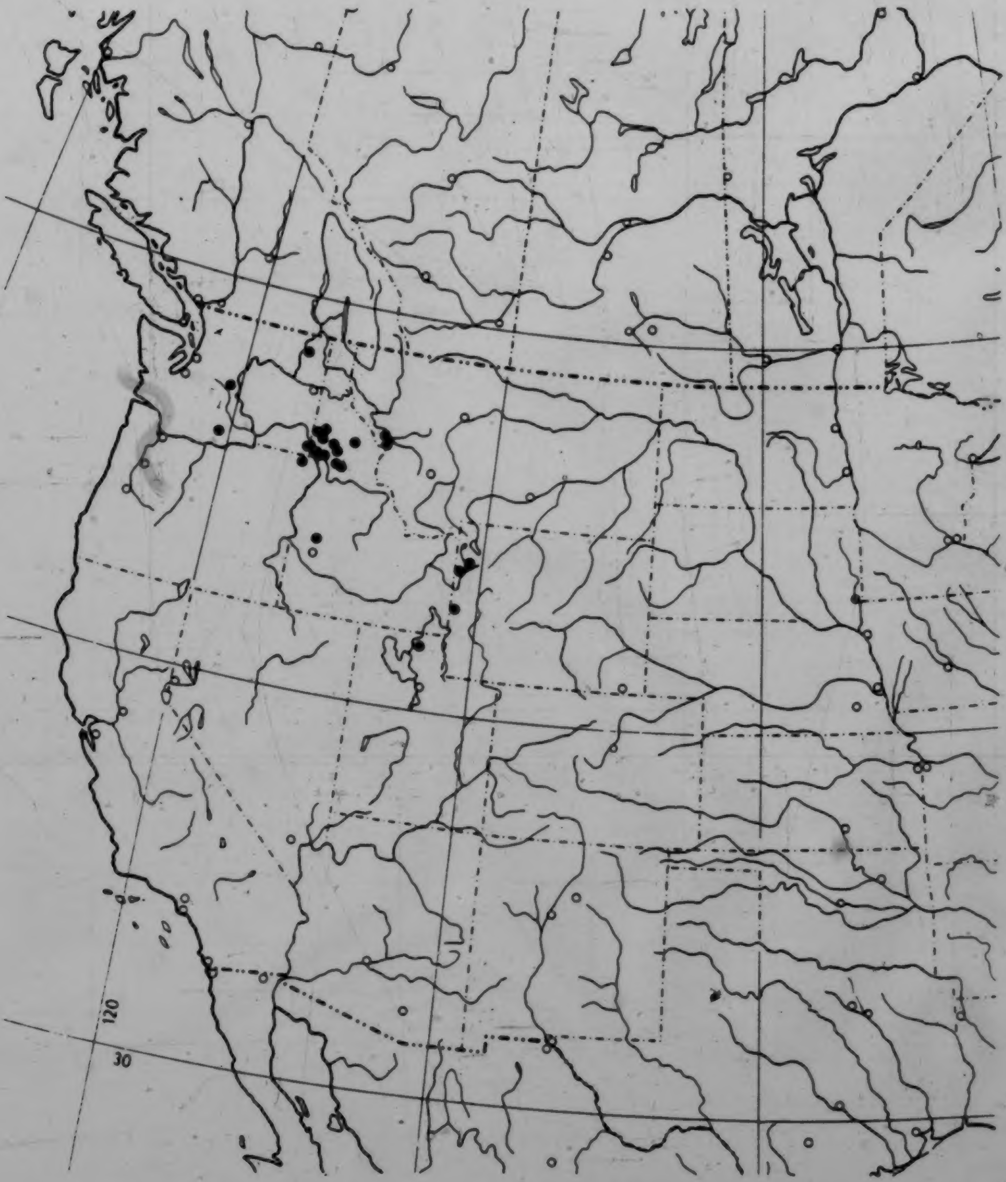
Leaves 60 to 80 mm long and 23 to 21 mm wide, when fully matured, margins regularily serrate-crenate , pubescence generally over 0.5 mm long, seeds about 4.1 mg, caruncle less than 30% of its length; pollen 34 to 37 um in diameter; 2N = 36, 48.

Occurs in the Rocky Mountains of the Idaho panhandle and adjacent Washington and Montana, with a few distant populations in western Wyoming and northern Utah (Figure 22). Found at lower elevations of 900 to 1200 m. The flowering period was from late April to early or mid June.

The collection number of the type specimens appears to have been misprinted at the time of publication as 3106 instead of the 3156 indicated on the type specimen which was from the correct location and on the correct date. The specimen at UC of the same collection without a collection number must be referred to here as well since it was collected at the same time and place.

Beginning with Piper and Beattie (1914), *flavovirens* has been treated as synonymous with *V. praemorsa* subspecies *major* by all authors. Annotations on herbarium sheets by M. S. Baker and J. Clausen indicated they may have considered reassigning it to variety or subspecies level. No such combination was ever published.

Figure 22. Distribution of *Viola praemorsa* Dougl. ex Lindl. subspecies *flavovirens* (Poll.) Fabijan.



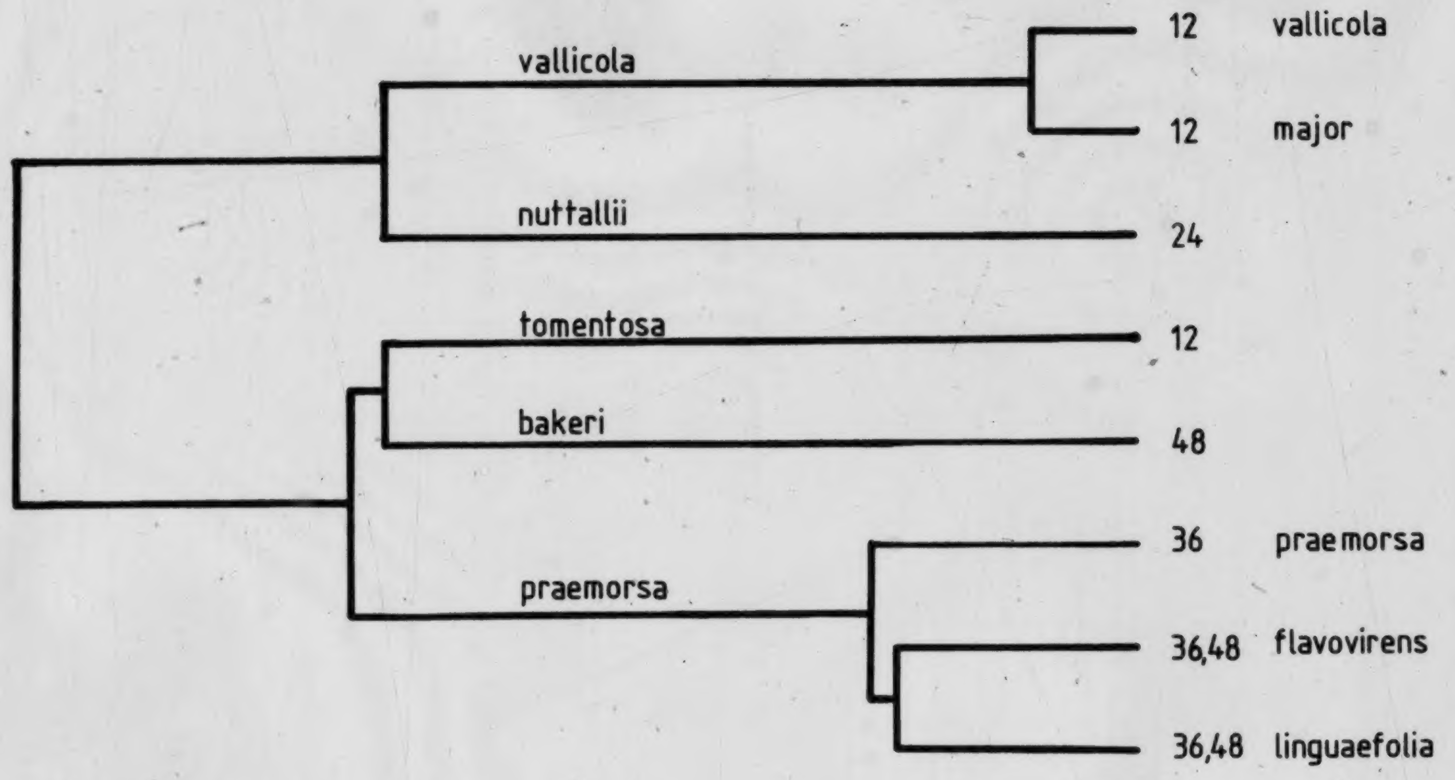
IV. DISCUSSION

This investigation of the *Viola nuttallii* complex resulted in the recognition of eight taxa (five species and three subspecies) based on their differences in ploidy level, morphological attributes and flavonoid chemistry: *V. vallicola*, 2N = 12; *V. nuttallii*, 2N = 24; *V. praemorsa*, 2N = 36, 48; *V. bakeri*, 2N = 48; and *V. tomentosa*, 2N = 12. Departures from the most recent, detailed analysis (Clausen, 1964) include the recognition of two subspecies of *V. vallicola*: *vallicola and major*; *V. praemorsa* is envisaged as embracing a wide range of morphological and chemical diversity the extremes of which are recognized as the subspecies *praemorsa*, *flavovirens*, and *linguaefolia*. In the absence of morphological or geographical differences between *V. linguaefolia* and *V. praemorsa* subspecies *major* and *oregona*, taxa recognized by Clausen (1964), these are included in subspecies *linguaefolia*. This treatment is supported by cytological and chemical evidence. A diagramatic representation of the taxonomic relationships among the members of the Nuttallianae is given in (Figure 23).

The *V. nuttallii* complex has long been recognized as a polyploid series with a basic chromosome number of x = 6. Chromosome counts of 2N = 12, 24, 36 and 48 obtained in this study confirm all previous counts reported for this complex (Table 5). It is known that in related species, cell sizes increase with increased ploidy level (Sax and Sax, 1937). This principle was confirmed for the *V. nuttallii*complex, in which pollen grain diameter is correlated with an increase in chromosome number (Figure 2). Pollen size can, therefore be employed in determining ploidy level in this complex, and may be of considerable utility in any future investigation of these taxa.

Hybrids had been reported in the literature by both Clausen (1964) and Davidse (1976). These included hybrids between subspecies *linguaefolia* and *V. utahensis* (Purpureae) which produced an n = 18 individual; and between *linguaefolia* and *V. douglasii* (Chrysanthae). This latter hybridization was believed by Clausen (1964) to have given rise to what Baker (1949c) considered to be *V. praemorsa* subspecies *arida*. Hybridizations between *V. tomentosa* and various members of the Purpureae and Chrysanthae subsections have also been found (Table 2). The presence of these reported hybrids indicates a close relationship between the *praemorsa*, *tomentosa*

Figure 23. Taxonomic relationship of the members of the *Viola nuttallii* complex.


vallicola
nuttallii
tomentosa
bakeri
praemorsa
12 vallicola
12 major
24
12
48
36 praemorsa
36,48 flavovirens
36,48 linguaefolia

species and other subsections in the Chamaemelanium.

A new hybrid (Table 5) was discovered between subspecies *linguaefolia* (2N = 36) and *V. purpurea* (2N = 12), subsection Purpurea, which grew intermixed at a location in California. The hybrid had a mitotic chromosome complement of 2N = 28 and pollen viability was estimated at only 7%, indicating that it was probably sterile.

No hybrids involving *V. vallicola* or *V. nuttallii* were observed nor have any been reported.

Prior to this investigation, very little was known about the flavonoid chemistry of *Viola*. The genus had been reported to contain the following flavones and flavonols: quercetin, kaempferol, luteolin, apigenin, violanthin, vitexin, saponaretin, orientin, and isoorientin (Bate-Smith, 1962; Wagner *et al.*, 1972; Gibbs, 1974; and Harborne, 1975). The only taxonomic work within the genus involving flavonoids, used only flavonoid profile analysis (Stebbins *et al.*, 1963 and McPherson, 1972).

The present study identified luteolin aglycone, oxygenated glycosides of apigenin, luteolin, kaempferol, and quercetin; a 6-methyl apigenin glycoside; and, a 6-methyl kaempferol glycoside. The most common glycosides were rutinose, glucose, galactose, rhamnose and arabinose (Figure 11 and Table 6). Further identification of the unknown compounds is expected to reveal the presence of some of the other previously reported flavonoids. Their presence is suspected from the comparison of Rf values with those reported in the literature (Harborne, 1967; Mabry *et al.*, 1970; and Jay *et al.*, 1975).

Despite the incomplete identification of all extracted flavonoids, careful examination of flavonoid spot patterns on two-dimensional paper chromatography and comparison of the major types of aglycones proved to be helpful in evaluating taxonomic boundaries and relationships among taxa. Flavonoid profiles have proven useful in related work in *Viola* subsection Purpurea (Stebbins *et al.*, 1963) where the ancestry of amphiploids and introgressants were investigated.

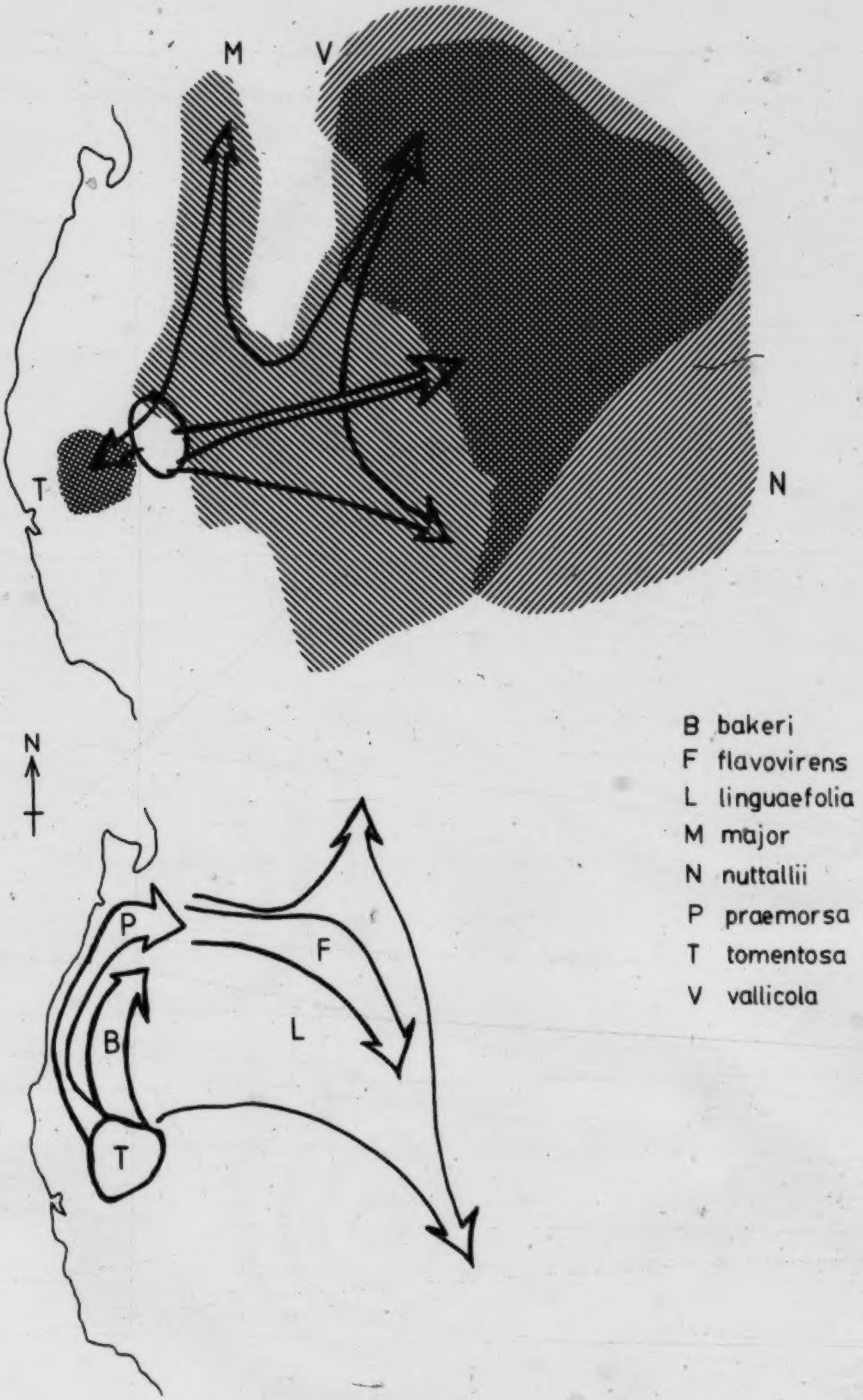
In the *V. nuttallii* complex, it was possible to correlate flavonoid pattern with ploidy level, morphological features, and distributions. The phytogeographic and phylogenetic implications of flavonoid analysis will be discussed for each taxon. The proposals for the evolutionary history of the members of the Nuttallianae are summarized in Figure 24.

The chromosome number of 2N = 12 for *V. vallicola* for 30 populations from British Columbia, Alberta and Saskatchewan (Table 5) supplements 19 previous counts (Davidse, 1976) from Idaho, Nevada, and Utah. Together, these reports cover a large portion of the range of this taxon. Only the easternmost populations in the range remain to be examined.

Viola vallicola specimens contained a total of eleven different flavonoid compounds: attributes 1, 2, 3, 4, 5, 6, 7, 9, 10, 11 and 29 (Table 7). Examination of chromatographic profiles revealed the presence of two distinct flavonoid races in this species plus a third pattern which appeared to be intermediate. None of these patterns could be correlated with any morphological feature examined in this study (Appendix 3), they could, however, be correlated with geographic occurrence (Figure 16). Subspecies *vallicola* contained primarily (frequency of 50% or greater) apigenin derivatives , compounds 2 and 3 (Table 7), and was found in Alberta and Saskatchewan. Subspecies *major* was found to contain predominantly kaempferol glycosides, compounds 1 and 9, and occurred in the interior of British Columbia and Oregon. A number of specimens from the Great Basin regions of Idaho and Nevada were found to be intermediate; they lacked compounds 2 and 9, but possessed compounds 1 and 3, and also contained two novel compounds, 5 and 10.

The recent elucidation of the general biosynthetic pathways of flavonoids in a number of higher plant taxa (Giannasi, 1978) indicate that the flavone apigenin represents one biosynthetic step from the precursor flavanone while kaempferol represents two biosynthetic steps: from flavanone to flavanonol to flavonol. Subspecies *major*, which possessed flavonols, may be considered further removed from the ancestor than subspecies *vallicola* because of its possession of these more complex flavonoid characters. The intermediates could be considered as either ancestral to one, or both subspecies, or as introgressive hybrids between two ancestral chemical forms. It would be impossible to prove which of these possibilities was true until specimens from the entire range of *V. vallicola* were examined for their flavonoid profiles and all compounds

Figure 24. A proposal for the evolutionary history of the *Viola nuttallii* complex.


M
V
T
N
N
P
F
B
L
T
B bakeri
F flavovirens
L linguaefolia
M major
N nuttallii
P praemorsa
T tomentosa
V vallicola

were positively identified.

The Great Basin region, lying in the area of continuous distribution across the continental divide, could represent the point where one subspecies migrated across giving rise to the other; the area of dispersal of the species resulting in two chemical variants; or the area of overlap of two ancestral chemical forms with the opportunity for introgression. The hypothesis of genetic flow across this region is supported by the apparent filtering of biosynthetic capabilities across it (Table 7). Compound 2, found at 96% frequency in subspecies *vallicola*, was present in 17% of the intermediates and 0% of subspecies *major*. Compound 1, present in 100% of the *major* specimens and intermediate specimens, was found in one specimen of subspecies *vallicola*. Compound 10 was present in only 50% of the intermediates, and was present in 17% of the subspecies *major* specimens. The only unique compound present in the intermediate was compound 5. Until all compounds are identified, this interpretation remains speculative.

Alston and Turner (1962) used flavonoid patterns to investigate introgression in natural hybrids and concluded that progeny resulting from introgressive hybridization rarely contain unique compounds. More recent work (Levy and Levin, 1971) indicates that novel compounds are produced in hybrids. The novel compounds were the probable result of repression or suppression of glycosylation in the hybrid, which would result in less highly glycosylated flavonoids than those found in either of the parents. The situation for *V. vallicola* could be clarified simply by examining the flavonoids present in artificial hybrids between the two subspecies.

Three phylogenetic senarios are proposed. With the gradual, continuous uplift of the Rocky Mountains during the Quaternary, *V. vallicola* or its ancestral form became split into eastern and western populations. The Oligocene cooling trend also resulted in gradual migration of vegetation to lowland areas (Tidwell *et al.*, 1972). The three major glaciations of the Pleistocene covered the eastern Sierra Nevadas, Cascades, northeastern Utah, the Uinta Mountains, and parts of the Great Basin, as well as the Rocky Mountains and most of western Canada (Tidwell *et al.*, 1972). The Intermountain region however, was semiarid during these maximum advances perhaps allowing for the meeting of the two subspecies. The consequence was introgressive hybridization with disruption of flavonoid biosynthesis at the genetic or regulatory levels (Levy and Levin, 1971)

If before the Pleistocene glaciations *V. vallicola* or its ancestor was found on only one side of the Rocky Mountains, migration south to the Great Basin area during glacial maxima, may have afforded it an opportunity to migrate across the continental divide and expand its range concomitent with an evolutionary change in flavonoid biosynthesis.

The third proposal, favored by the author, suggests that isolation during post-glacial migration northward on either side of the mountains could account for the differentiation of two subspecies. The loss of flavonoid synthesizing capability for flavonols in subspecies *vallicola* is incomplete because some specimens appeared to accumulate kaempferol and quercetin derivatives. Genetic isolation, therefore, cannot be absolute even in the northern most portion of the range. Further flavonoid surveys would help to establish whether the subspecies were a purely northern phenomenon or represent an east-west differentiation.

Twelve populations of *V. nuttallii* from Alberta and Saskatchewan were found to possess somatic chromosome counts of 2N = 24 (Table 5), confirming the two previous counts for this taxon (Baker, 1949c; Clausen, 1964). One of these counts was from a transplant of nursery stock from Colorado; the other from buds fixed by W. C. McCalla (*7355*, ALTA) from a population in Calgary, Alberta.

Viola nuttallii contains one flavone (3, an apigenin derivative), several flavonols (1 and 4, kaempferol 3-O diglycosides; and 6, 7, 8, quercetin 3-O and 3,7-O diglycosides) and other flavonoids (8, 10, 12, at low frequencies; see Table 7). Morphologically, *V. nuttallii* is most similar to *V. vallicola*, differing principally in leaf shape, and seed size and colour (Figure 12, Appendices 2 and 3). Chemically, *nuttallii* is most similar to *V. vallicola* subspecies *vallicola*, with 56% of their flavonoids in common and *V. tomentosa*, with 50% of their flavonoids common (Table 7) and which it approaches in leaf basal angle and seed colour.

V. vallicola and *V. nuttallii* are sympatric east of the Rocky Mountains (Figures 14 and 16) and some populations in Alberta and Saskatchewan were observed to grow in close proximity. No hybrids were observed and none have been reported in the literature.

V. nuttallii was thought to be an autotetraploid of *V. vallicola* by Baker (1957), while Clausen (1964) speculated that it might have originated from an as yet undiscovered diploid race of *V. nuttallii*. No such diploid has been discovered. Morphological and chemical evidence presented in this study suggest the possibility that *V. nuttallii* is an allotetraploid of *V. vallicola* and *V. tomentosa* (Table 7).

Levy (1976) found that colchicine-induced autotetraploids of *Phlox* exhibited novel glycoflavonoids, loss of diploid glycoflavonoids and deregulation of glycoflavonoid biosynthesis. All of these features can be observed in the differences in leaf flavonoids between *V. nuttallii* and *V. vallicola* subspecies *vallicola*. *V. nuttallii* did not possess apigenin 7-O diglycoside (2) but did possess novel flavonoids 8 (quercetin 3-O monoglycoside), 10, and 12. Deregulation of flavonoid biosynthesis was found for compounds 1, 4, 6 and 7, both in terms of frequency and in relative intensity of spot patterns on paper chromatographs (Appendix 1).

The same comparison of lost and novel compounds, and the deregulation of flavonoid production was observed between *V. tomentosa* and *V. vallicola*. They are not sympatric at the present time, but their ranges may have overlapped at some time in the past. With the increasing Pleistocene aridity in the Great Basin and California, *nuttallii* (and *vallicola*) is proposed to have become adapted to more xeric conditions than *tomentosa*, and moved into the lowland and prairie environments (Figure 24A). *V. nuttallii* is not known west of the Rocky Mountains, it's range overlaps only with *V. vallicola* subspecies *vallicola*. The ecological requirements of *V. nuttallii* are very similar to those of *V. vallicola* and may lead one to expect them to have the same distributions. However, *V. nuttallii* has a maximum elevational range of 2400 m, not as high as *V. vallicola*, and may have been unable to migrate westward over the mountains.

Viola tomentosa, a diploid, possessed apigenin (2 and 3), kaempferol (1) and quercetin (6, 7, 8) derivatives, and flavonoid 24 (Table 7). It had 38% of its flavonoids in common with the diploid *V. vallicola*, and 50% in common with the tetraploid *V. nuttallii*. This suggests that these taxa are related in some way. The distributions of *V. vallicola* and *V. nuttallii* do not overlap with *V. tomentosa*, which occurs in upland forest areas in the Sierra Nevada. Their ranges may have diminished in recent times due to the Pliocene uplift of the Sierra Nevada and Cascade Ranges (Jardin and McKenzie, 1972) in conjunction with the increasing aridity and summer drought conditions on the west coast (Chaney, 1947).

A chromosome count of 2n = 48 (Table 5) for *V. bakeri* from Trout Lake, Washington, is the first for this taxon outside of California (Baker, 1949a). *V. bakeri* was most similar to *V. tomentosa*, whose range it overlaps in the Sierra Nevada (Figure 19). No tetraploid intermediate is known in that range, nor does there appear to be a suitable hexaploid with which it could have hybridized.

V. bakeri and *V. tomentosa* have only four (24%) of their flavonoids in common; three of these are quercetin derivatives (Table 7). The unique feature of *bakeri* (and *praemorsa*) specimens is the presence of a diverse array of different flavonoids and the presence of luteolin derivatives (11, 13, 15, and 17). The biosynthetic capability to produce these new flavonoids may be due to the effects of past (unknown) polyploidy or may have been introduced through hybridization with species in the closely related subsections Purpureae and Chrysanthae (Table 2).

V. bakeri occurs in the Sierra Nevada and Coastal ranges and occupies areas which also overlap with the subspecies of *V. praemorsa*. That *V. bakeri* possesses no compounds not found in *V. praemorsa* is a strong indication of a close phylogenetic relationship, perhaps through a common ancestor. The ancestral flavonoid pattern must then have included compounds 6, 7, 8, 11, 13, 14, 15, 16, 17, and 22, apigenin, luteolin, kaempferol and quercetin derivatives. It is also possible that hybridizations may occur between *bakeri* and octoploid *linguaefolia* and *flavovirens* in their regions of overlap in central Washington and southern Oregon and northern California. These regions contain a number of examples of possible hybrids such as subspecies *oregona* Baker (1957) in southern Oregon. TAXMAP analysis three (Figure 13, Appendix 4) revealed that though specimens of *oregona* were morphologically clustered with *linguaefolia*, they occupied a position intermediate between *bakeri* and *linguaefolia*.

V. praemorsa is composed of three subspecies which represent recognizable extremes in the range of morphological variation present in the species. As outlined in the results these variations existed in cytological, morphological, and chemical attributes. These taxa were found to contain a vast array of different flavonoids, up to 20 in one taxon, in comparison to diploid and tetraploid taxa (Table 7). One strikingly unique feature of this flavonoid profile was the presence of a new aglycone base, luteolin. The four identified luteolin derivatives (11, 13, 15, and 17) were prominent in the profiles of most specimens of *V. praemorsa* and *V. bakeri*.

Chromosome counts of 2N = 36 from two populations of *V. praemorsa* subspecies *praemorsa* in Washington and California, including a topolectotype (*DF 414*), of subspecies *praemorsa* (Table 5) confirm previous reports (Gershoy, 1934; Baker, 1949c and 1957; and Clausen, 1964). Subspecies *praemorsa* is a Pacific coast entity whose range extends inland in the region of the Columbia River and again along the Oregon California boundaries (Figure 20). In both of these inland areas it overlaps the ranges of *V. vallicola* subspecies *vallicola*, *V. bakeri*, subspecies *flavovirens*, and subspecies *linguaefolia*.

Subspecies *flavovirens* represents a morphologically distinct taxon which occurs in isolated pockets in the Rocky Mountains within the range of subspecies *linguaefolia* (Figure 22). Counts of 2N = 36 and 48 are the first reported chromosome numbers for this taxon (Table 5).

The previous reports of chromosome counts for *linguaefolia* of n = 18 (Clausen, 1964) and n = 24 (Davidse, 1976) were both confirmed for ten populations in Washington, Oregon, California, Idaho, Montana, and Alberta (Table 5). In all cases pollen viability estimates were high (80 to 95% , Figure 2). Clausen and Baker (Baker, 1949c) first reported a chromosome count of 2N =36 for two populations of *linguaefolia* from Idaho and Utah (Clausen, 1964), and 2N = 48 for four populations of *major sensu* Baker. Even though "the morphological characters are not highly distinct," Clausen retained *linguaefolia* at species level, because of his belief of its unique chromosome number. Davidse (1976), finding only counts of 2N = 48, preferred to consider *linguaefolia* in synonymy with *V. praemorsa* subspecies *major sensu* Baker. In nine populations from

Utah, Nevada, and Idaho, all plants had n = 24 with regular meiosis and high pollen fertility. No reliable morphological characteristics or geographic distributions could be correlated with ploidy level (Figure 13, Appendix 4), so it was not considered justifiable to recognize other infraspecific taxa.

Subspecies *linguaefolia* is certainly the most morphologically heterogenous taxon in the species. Considering the areas of sympatry, and chromosome complement similarities, assumptions of genetic isolation between the three *praemorsa* subspecies are not justified. In fact, the possibility of hybridization between these taxa and species of other sections or subsections (Table 2, Table 5) have been reported.

Subspecies *linguaefolia* appears to contain two flavonoid forms (Table 7). The first, and apparently most widespread, resembles the general diverse pattern as defined above. The second is a much reduced profile containing primarily kaempferol (1 and 9) and quercetin (6, 7, 8, and 27) derivatives. The ubiquitous luteolin and apigenin derivatives of *bakeri* and *praemorsa* were completely absent in these specimens, which contained only seven flavonoids including three novel compounds (1, 10 and 27), but did not display any unique morphological features (Figure 13). The possibility exists that these individuals represent ecologically isolated populations which subsequently underwent a reduction of flavonoid profile (Wolf and Denford, 1983). More complete flavonoid surveys may reveal geographic isolation at high elevations or high latitudes at the northeastern edge of the range of *praemorsa*. Certainly it was impossible to establish any correlations based on the meager sampling of only four populations,though these were all collected on or near the continental divide (Table 4).

The diversity of flavonoids present in the leaves of *V. bakeri* and *V. praemorsa*, the extent of morphological variation and the existence of hybrids lead to the hypothesis that these taxa are the most recent in the *V. nuttallii* complex. In only a few scattered populations of *linguaefolia* was it possible to observe flavonoid reduction (Wolf and Denford, 1983) in the elimination of all flavone biosynthesis.

Glaciation events during the Pleistocene likely had an effect on speciation in the Nuttallianae by drastically effecting the the climatic conditions on the west coast. At some point in the evolutionary history of these taxa, two lineages arose. *V. vallicola* and V. *nuttallii*, probably the oldest taxa in the Nuttallianae, differentiated and became established in the more xeric habitats of the valley bottoms in the rainshadows of the Cascade and Rocky Mountains. They can be differentiated from the other Nuttallianae by their minute puberulence, nearly entire leaf margins, seeds with flattened caruncles which cover the funiculus and exceed it in length, and reduced flavonoid profiles consisting of apigenin , kaempferol and quercetin derivatives.

V. tomentosa, *V. bakeri*, and *V. praemorsa* became adapted to the more mesic forest habitats of the coastal and montane forests. These taxa, though of diverse morphology, generally possess long pubescence, seeds with globose caruncles which do not cover or exceed the funiculus in length, and diverse flavonoid profiles consisting of apigenin, kaempferol and luteolin compounds. Hybridization between these taxa and taxa of the subsections Chrysanthae and Purpurea indicated that the phylogenetic relationship of the Nuttallianae to other subsections is through these species, as proposed by Clausen (1929). Diploids, tetraploids, hexaploids and octoploids alike have expanded into previously glaciated areas.

Ploidy level in the Nuttallianae has been correlated with morphology, flavonoid chemistry, and phytogeographic data. A classification has been formulated based on these data, which proposes that the *nuttallii* complex be split into eight taxa - five species and three subspecies. This classification, which takes into account the polyploid nature of the complex, attempts to incorporate events of genetic isolation, hybridization, and introgression into the evolutionary history of the group. Correlation of some morphological characters and the presence of different flavonoid profiles support the speculation that two groups of species differentiated from a single ancestral complex. The first group includes *V. vallicola* and *V. nuttallii*, two of the oldest members of the Nuttallianae, which represent homogenous species of wide-ranging distribution. The second group comprises the remaining three species in the Nuttallianae. *V. tomentosa* and *V. bakeri*, also of homogenous morphology, display much more restricted distributions. *V. praemorsa* surely represents the most difficult systematic problem remaining in the complex due to its heterogenous nature and widespread distribution. Hypothetical relationships advanced in this discussion await more in depth analysis into the morphological, biochemical, and cytological aspects of *V. praemorsa* as well as breeding experiments for confirmation.

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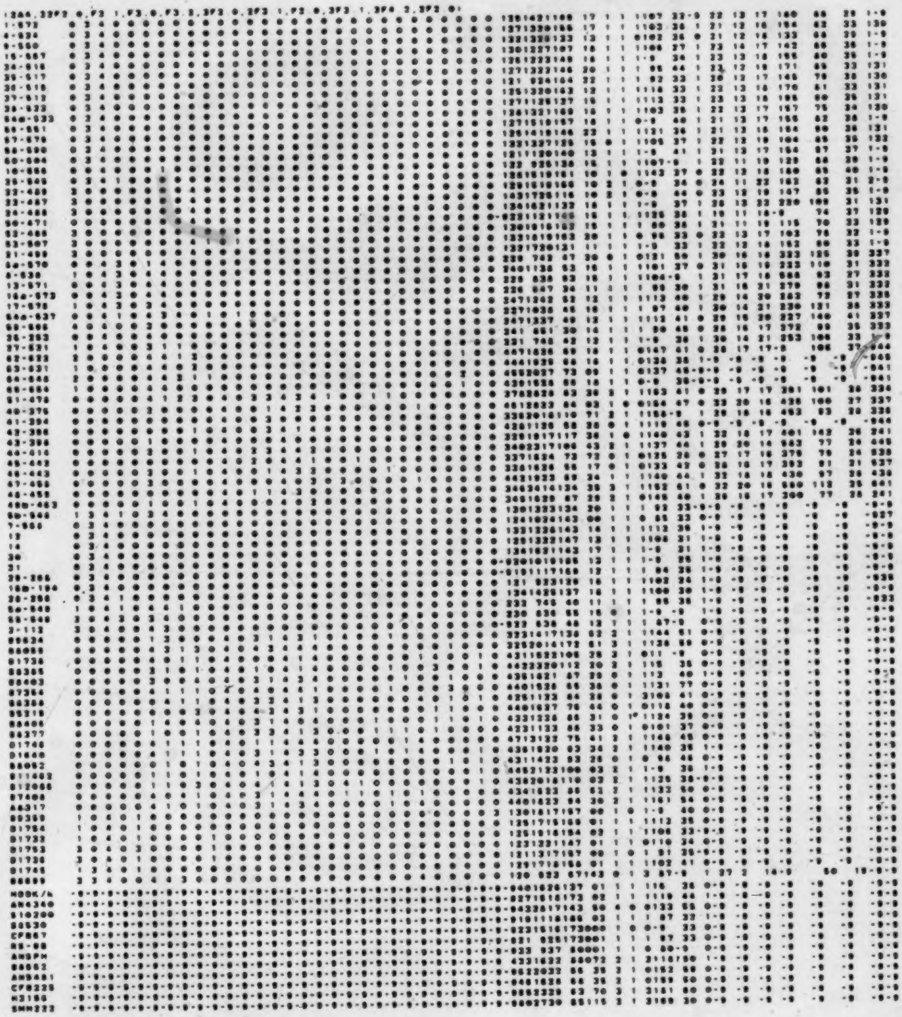
VI. APPENDIX 1. Data for TAXMAP analyses.

The raw data for all 95 OTUs (Table 4) and 50 attributes (Table 3), is listed as arranged for the TAXMAP data file. The first line represents the FORTRAN format statement.



VII. APPENDIX 2. TAXMAP Analysis 1.

This analysis was performed using the author's collection of 59 OTUs scored for 50 attributes including flavonoid chemistry, cytological and morphological characteristics. TAXMAP was ran using an equally weighted analysis which does not take into account the relative information content of each attribute.

The TAXMAP print out includes the complete proximity table of the relative distance of each OTU from every other OTU. The nearest neighbour table lists the eight closest neighbours of each OTU in order with their distances. The table summarizing the cluster anaysis lists the clusters, cluster members, and and single-membered clusters. The Mapping Aids includes the intercluster distances as center-to-center distances and minimum intercluster discontinuities.

```
PROXIMITIES (AS DISTANCE * 1000), COMPUTED FOR ATTRIBUTES   1 TO  50
    1     2  31    3  61    4  47    5  30    6  33    7  43    8  25    9  39   10  28   11  54   12  30   13  60   14  39   15  81   16  79
    1    17 217   18 200   19 166   20 132   21 165   22 162   23 140   24 291   25 265   26 271   27 269   28 269   29 277   30 255   31 211
    1    32 214   33 383   34 337   35 411   36 279   37 536   38 470   39 314   40 364   41 340   42 468   43 379   44 426   45 374   46 404
    1    47 330   48 107   49  69   50  41   51  24   52  24   53  30   54  26   55  23   56  49   57 271   58 292   59 213    3  88    4  47
    2     5  27    6  28    7  24    8  27    9  42   10  21   11  51   12  25   13  54   14  37   15  85   16  99   17 240   18 221   19 168
    2    20 123   21 138   22 191   23 134   24 287   25 290   26 275   27 266   28 274   29 272   30 282   31 236   32 221   33 420   34 350
    2    35 435   36 301   37 562   38 493   39 323   40 405   41 348   42 471   43 400   44 431   45 383   46 433   47 351   48 104   49  97
    2    50  41   51  20   52  43   53  36   54  24   55  41   56  42   57 278   58 289   59 235    4  99    5  83    6  87    7  96    8  87
    3     9  91   10  90   11  66   12  98   13  93   14  95   15 121   16 106   17 194   18 155   19 219   20 181   21 200   22 147   23 181
    3    24 281   25 275   26 308   27 304   28 294   29 311   30 268   31 229   32 258   33 364   34 351   35 380   36 248   37 545   38 433
    3    39 374   40 393   41 403   42 519   43 341   44 416   45 417   46 433   47 331   48 157   49  50   50  78   51  78   52 100   53  98
    3    54 105   55  96   56  85   57 310   58 338   59 230    5  31    6  36    7  49    8  46    9  22   10  35   11  69   12  40   13  39
    4    14  39   15  87   16 110   17 266   18 206   19 178   20 141   21 177   22 210   23 146   24 260   25 270   26 254   27 253   28 249
    4    29 243   30 244   31 205   32 185   33 397   34 317   35 415   36 281   37 549   38 487   39 310   40 359   41 328   42 443   43 389
    4    44 408   45 361   46 417   47 324   48 112   49  95   50  46   51  39   52  46   53  63   54  56   55  43   56  51   57 254   58 264
    4    59 226    6  19    7  30    8  21    9  20   10  19   11  49   12  29   13  41   14  30   15  74   16  92   17 241   18 204   19 159
    5    20 119   21 151   22 192   23 131   24 264   25 270   26 255   27 248   28 253   29 248   30 260   31 218   32 201   33 416   34 341
    5    35 431   36 295   37 551   38 489   39 321   40 391   41 336   42 468   43 393   44 420   45 365   46 432   47 338   48 102   49  84
    5    50  45   51  20   52  37   53  35   54  28   55  29   56  35   57 260   58 271   59 234    7  39    8  26    9  22   10  24   11  64
    6    12  35   13  34   14  43   15  67   16  93   17 233   18 201   19 154   20 126   21 147   22 196   23 130   24 276   25 270   26 260
    6    27 250   28 254   29 253   30 258   31 227   32 196   33 420   34 350   35 435   36 301   37 554   38 494   39 320   40 389   41 343
    6    42 466   43 396   44 421   45 365   46 427   47 345   48 105   49  88   50  41   51  25   52  46   53  41   54  36   55  39   56  40
    6    57 267   58 282   59 238    8  35    9  46   10  28   11  49   12  31   13  60   14  36   15  88   16 101   17 254   18 227   19 183
    7    20 115   21 148   22 196   23 149   24 284   25 306   26 287   27 275   28 288   29 274   30 287   31 243   32 231   33 439   34 359
    7    35 454   36 310   37 566   38 509   39 342   40 420   41 355   42 491   43 419   44 450   45 396   46 444   47 359   48  97   49 102
    7    50  45   51  23   52  40   53  30   54  19   55  33   56  43   57 290   58 287   59 234    9  30   10  22   11  51   12  26   13  48
    8    14  32   15  77   16  87   17 224   18 212   19 161   20 113   21 148   22 177   23 126   24 283   25 277   26 261   27 257   28 257
    8    29 259   30 267   31 229   32 207   33 415   34 344   35 429   36 300   37 551   38 489   39 320   40 399   41 338   42 471   43 400
    8    44 425   45 371   46 434   47 338   48 100   49  83   50  44   51  21   52  34   53  22   54  19   55  39   56  39   57 269   58 279
    8    59 242   10  31   11  68   12  35   13  35   14  33   15  72   16  97   17 249   18 202   19 158   20 126   21 166   22 204   23 132
    9    24 262   25 262   26 245   27 244   28 250   29 240   30 248   31 207   32 188   33 411   34 336   35 424   36 298   37 546   38 485
    9    39 315   40 375   41 334   42 463   43 393   44 411   45 359   46 423   47 340   48 109   49  80   50  43   51  31   52  41   53  44
    9    54  42   55  38   56  43   57 255   58 263   59 227   11  51   12  31   13  49   14  37   15  84   16  95   17 240   18 218   19 167
   10    20 124   21 143   22 184   23 138   24 282   25 285   26 269   27 265   28 263   29 265   30 270   31 227   32 211   33 423   34 350
   10    35 438   36 306   37 558   38 496   39 323   40 395   41 343   42 466   43 396   44 423   45 375   46 431   47 344   48 104   49  89
   10    50  36   51  21   52  41   53  36   54  28   55  44   56  45   57 275   58 287   59 243   12  48   13  83   14  52   15 119   16 115
   11    17 216   18 192   19 197   20 148   21 177   22 158   23 163   24 249   25 318   26 303   27 291   28 299   29 285   30 295   31 257
   11    32 246   33 391   34 313   35 409   36 254   37 577   38 469   39 346   40 429   41 358   42 497   43 376   44 401   45 404   46 459
   11    47 314   48 123   49  77   50  80   51  46   52  48   53  63   54  56   55  64   56  70   57 304   58 306   59 279   13  53   14  22
   12    15  85   16  95   17 245   18 217   19 166   20 119   21 160   22 185   23 134   24 282   25 284   26 269   27 269   28 273   29 259
   12    30 270   31 227   32 219   33 409   34 332   35 425   36 297   37 548   38 485   39 308   40 396   41 327   42 463   43 394   44 424
   12    45 372   46 420   47 341   48  97   49  91   50  49   51  24   52  26   53  41   54  32   55  40   56  41   57 280   58 281   59 247
   13    14  48   15  69   16 100   17 251   18 196   19 173   20 137   21 166   22 208   23 139   24 269   25 273   26 261   27 264   28 253
   13    29 251   30 252   31 227   32 204   33 422   34 349   35 434   36 298   37 556   38 498   39 329   40 385   41 349   42 475   43 389
   13    44 405   45 372   46 439   47 347   48 117   49  98   50  48   51  44   52  55   53  58   54  65   55  58   56  45   57 277   58 267
   13    59 248   15  74   16  92   17 250   18 210   19 167   20 116   21 167   22 198   23 135   24 272   25 275   26 259   27 256   28 262
   14    29 246   30 254   31 215   32 206   33 414   34 334   35 424   36 300   37 547   38 481   39 316   40 394   41 327   42 471   43 402
   14    44 419   45 368   46 424   47 344   48 103   49  84   50  48   51  27   52  30   53  31   54  28   55  33   56  46   57 267   58 271
   14    59 237   16 136   17 221   18 189   19 149   20 107   21 143   22 192   23 123   24 253   25 236   26 212   27 219   28 215   29 223
   15    30 248   31 214   32 170   33 385   34 320   35 404   36 254   37 515   38 471   39 303   40 376   41 335   42 454   43 384   44 400
   15    45 345   46 412   47 325   48 134   49 120   50  76   51  82   52  93   53  78   54  89   55  79   56  77   57 236   58 249   59 185
   16    17 240   18 210   19 231   20 185   21 215   22 203   23 189   24 322   25 276   26 306   27 312   28 305   29 299   30 261   31 240
   16    32 275   33 431   34 405   35 440   36 348   37 567   38 447   39 383   40 415   41 346   42 530   43 413   44 483   45 429   46 439
   16    47 408   48 151   49  95   50 109   51  92   52  93   53  99   54 116   55 128   56  87   57 322   58 321   59 264   18  82   19 132
   17    20 168   21 137   22  76   23 131   24 369   25 331   26 388   27 372   28 355   29 375   30 283   31 314   32 333   33 299   34 317
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   17    50 249   51 241   52 246   53 265   54 242   55 252   56 245   57 383   58 383   59 265   19 122   20 153   21 123   22  63   23  90
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19   44 398  45 354  46 410  47 324  48 226  49 223  50 178  51 169  52 162  53 190  54 180  55 170  56 170  57 290  58 286
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22   50 201  51 192  52 184  53 216  54 239  55 241  56 201  57 369  58 368  59 260  24 329  25 304  26 308  27 305  28 290
23   29 303  30 260  31 273  32 234  33 320  34 285  35 365  36 268  37 490  38 474  39 308  40 378  41 330  42 445  43 393
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23   59 243  25 136  26 116  27 101  28 134  29 138  30 206  31 177  32 185  33 317  34 244  35 339  36 216  37 539  38 447
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43   59 415  45 234  46 300  47 170  48 446  49 423  50 416  51 426  52 417  53 446  54 407  55 406  56 426  57 395  58 425
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47   50 335  51 332  52 332  53 347  54 304  55 316  56 343  57 371  58 401  59 391  49 169  50 107  51  90  52  95  53 122
48   54 126  55 115  56  79  57 215  58 209  59 168  50  98  51  87  52 101  53  97  54 112  55 117  56 101  57 321  58 327
49   59 240  51  32  52  51  53  47  54  46  55  50  56  43  57 275  58 293  59 236  52  27  53  32  54  26  55  28  56  33
51   57 265  58 277  59 236  53  37  54  36  55  34  56  42  57 278  58 274  59 249  54  13  55  21  56  56  57 259  58 266
53   59 244  55  29  56  55  57 215  58 239  59 236  56  49  57 230  58 254  59 223  57 261  58 272  59 254  58  91  59 104
58   59 111   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0
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NEAREST NEIGHBOURS (DISTANCE *1000)

 1 1-572    v-a      1 55  23   1 51  24   1 52  24   1  8  25   1 54  26   1 10  28   1  5  30   1 53  30
 2 4-524    v-a      2 51  20   2 10  21   2  7  24   2 54  24   2 12  25   2  5  27   2  8  27   2  6  28
 3 9-550    v-a      3 49  50   1  3  61   3 11  66   3 51  78   3 50  78   3  5  83   3 56  85   3  8  87
 4 15-567   v-a      4  9  22   4  5  31   4 10  35   4  6  36   4 51  39   4 14  39   4 13  39   4 12  40
 5 34-516   v-a      5  6  19   5 10  19   5 51  20   5  9  20   5  8  21   2  5  27   5 54  28   5 12  29
 6 35-517   v-a      5  6  19   6  9  22   6 10  24   6 51  25   6  8  26   2  6  28   1  6  33   6 13  34
 7 36-519   v-a      7 54  19   7 51  23   2  7  24   7 10  28   5  7  30   7 53  30   7 12  31   7 55  33
 8 37-520   v-a      8 54  19   5  8  21   8 51  21   8 53  22   8 10  22   1  8  25   8 12  26   6  8  26
 9 38-523   v-a      5  9  20   4  9  22   6  9  22   8  9  30   9 51  31   9 10  31   9 14  33   9 13  35
10 54d-533  v-a      5 10  19   2 10  21  10 51  21   8 10  22   6 10  24   1 10  28  10 54  28   7 10  28
11 56-551   v-a     11 51  46  11 12  48  11 52  48   7 11  49   5 11  49   8 11  51   2 11  51  10 11  51
12 57-579   v-s     12 14  22  12 51  24   2 12  25   8 12  26  12 52  26   5 12  29   1 12  30   7 12  31
13 58-590   v-s      6 13  34   9 13  35   4 13  39   5 13  41  13 51  44  13 56  45  13 50  48   8 13  48
14 60-604   v-s     12 14  22  14 51  27  14 54  28   5 14  30  14 52  30  14 53  31   8 14  32  14 55  33
15 10-556   v-a      6 15  67  13 15  69   9 15  72  14 15  74   5 15  74  15 50  76  15 56  77   8 15  77
16 25-545   v-a      1 16  79  16 56  87   8 16  87  14 16  92  16 51  92   5 16  92  16 52  93   6 16  93
17 22-489   v-bc    17 22  76  17 18  82  17 23 131  17 19 132  17 21 137  17 20 168   3 17 194  17 49 195
18 23-487   v-bc    18 22  63  17 18  82  18 23  90  18 19 122  18 21 123  18 20 153   3 18 155  18 49 160
19 24-488   v-bc    19 23  46  19 21  70  19 20 106  19 22 111  18 19 122  17 19 132  15 19 149   6 19 154
20 50-471   v-o     20 23  72  20 21  76  19 20 106  15 20 107  20 51 111   8 20 113   7 20 115  14 20 116
21 52-486   v-bc    21 23  43  19 21  70  20 21  76  21 22  96  18 21 123  17 21 137   2 21 138  10 21 143
22 53-507   v-bc    18 22  63  17 22  76  22 23  83  21 22  96  19 22 111  20 22 126   3 22 147  22 49 155
23 21-499   v-bc    21 23  43  19 23  46  20 23  72  22 23  83  18 23  90  15 23 123   8 23 126   6 23 130
24 6a-570   n-a     24 58  86  24 27 101  24 26 116  24 57 119  24 28 134  24 25 136  24 29 138  24 31 177
25 8-538    n-a     25 28  72  25 26  79  25 27  80  25 58  82  25 59  91  25 57  97  25 31 104  25 29 108
26 13-571   n-a     26 27  63  26 28  69  25 26  79  26 57  83  26 58  86  26 29 114  24 26 116  26 59 130
27 16a-573  n-a     27 28  62  26 27  63  27 57  73  25 27  80  27 58  86  27 29  90  24 27 101  27 32 114
28 17-576   n-a     27 28  62  26 28  69  25 28  72  28 58  89  28 57  95  28 29 104  28 32 129  24 28 134
29 54a-537  n-a     29 57  89  27 29  90  28 29 104  29 58 107  25 29 108  26 29 114  29 32 116  24 29 138
30 59-596   n-s     30 59  73  30 31  90  25 30 118  30 58 137  30 32 142  28 30 146  29 30 148  30 57 160
31 26-253   n-a     30 31  90  25 31 104  31 59 112  27 31 122  31 32 124  31 57 126  29 31 139  26 31 141
32 27-531   n-a     32 57  90  27 32 114  29 32 116  32 59 120  31 32 124  28 32 129  30 32 142  32 58 153
33 62-622   1-1     33 36  77  33 35  78  33 34  89  33 59 216  33 57 264  18 33 266  25 33 268  22 33 270
34 63-631   1-m     34 36  60  33 34  89  34 35 132  34 59 219  34 57 220  34 58 240  26 34 243  28 34 244
35 64-648   1-a     33 35  78  35 36  91  34 35 132  35 59 225  35 57 286  25 35 293  31 35 298  30 35 299
36 65-654   1-a     34 36  60  33 36  77  35 36  91  36 59 163  36 57 193  25 36 199  28 36 203  32 36 211
37 51-478   m-w     37 44 257  37 42 264  37 40 299  37 46 301  37 43 303  37 38 305  37 39 313  37 47 316
38 40-375   m-w     38 45 189  38 40 259  38 43 270  38 44 271  38 47 273  38 46 279  38 41 281  37 38 305
39 41-394   m-w     39 41 186  39 42 199  39 47 221  39 46 222  39 45 250  39 54 251  39 40 251  39 43 252
40 42-396   b-w     40 45 209  40 43 212  40 44 230  40 47 241  40 42 249  40 46 251  39 40 251  38 40 259
41 43-398   m-w     41 47 173  39 41 186  41 45 209  41 46 235  41 54 254  41 42 263  40 41 276  41 43 276
42 44-414   p-w     42 46 192  39 42 199  42 47 235  42 44 246  40 42 249  42 45 249  42 43 253  41 42 263
43 45-442   m-o     43 44 162  43 47 196  40 43 212  43 46 217  39 43 252  42 43 253  38 43 270  41 43 276
44 46-443   o-o     43 44 162  44 47 170  40 44 230  44 45 234  42 44 246  37 44 257  38 44 271  41 44 285
45 47-458   a-o     38 45 189  45 47 202  40 45 209  41 45 209  44 45 234  42 45 249  39 45 250  34 45 254
46 48-459   m-c     42 46 192  43 46 217  39 46 222  41 46 235  40 46 251  46 47 274  38 46 279  45 46 293
47 49b-463  m-c     44 47 170  41 47 173  43 47 196  45 47 202  39 47 221  42 47 235  40 47 241  34 47 244
48 5b-548   v-a     48 56  79  48 51  90  48 52  95  12 48  97   7 48  97   8 48 100   5 48 102  14 48 103
49 7-568    v-a      3 49  50   1 49  69  11 49  77   9 48  80   8 49  83   5 49  84  14 49  84  49 51  87
50 12-565   v-a     50 51  32  10 50  36   2 50  41   1 50  41   6 50  41   9 50  43  50 56  43   8 50  44
51 14-566   v-a      2 51  20   5 51  20  10 51  21   8 51  21   7 51  23   1 51  24  12 51  24   6 51  25
52 30-547   v-a      1 52  24  12 52  26  51 52  27  14 52  30  52 55  34   8 52  34  52 54  36  52 53  37
53 31-276   v-a     53 54  13  53 55  21   8 53  22   1 53  30   7 53  30  14 53  31  51 53  32   5 53  35
54 29-268   v-a     53 54  13   8 54  19   7 54  19   2 54  24   1 54  26  51 54  26  10 54  28  14 54  28
55 16b-194  v-a     53 55  21   1 55  23  51 55  28   5 55  29  54 55  29   7 55  33  14 55  33  52 55  34
56 28-256   v-a     51 56  33   5 56  35   8 56  39   6 56  40  12 56  41  52 56  42   2 56  42   9 56  43
57 5a-549   n-a     27 57  73  26 57  83  29 57  89  32 57  90  57 58  91  28 57  95  25 57  97  57 59 104
58 55-546   n-a     25 58  82  26 58  86  27 58  86  24 58  86  28 58  89  57 58  91  29 58 107  58 59 111
59 2-112    n-a     30 59  73  25 59  91  57 59 104  58 59 111  31 59 112  32 59 120  27 59 125  26 59 130
```

```
MAP CLUSTER ANALYSIS:-NUTTALLIANAE, SEPTEMBER, 1983;  PLUS HERBARIUM SPECIMENS

(MINIMUM NUCLEUS 0.080, MAXIMUM DROP 0.0439 BOTH ARE 100% OF NORMAL)
EQUALLY WEIGHTED ATTRIBUTES

CLUS OTU DIST OTU  AVGOF  DROP FAR DIST FLAG NAME OF OTU
TER  NOS BEST BEST  NEW    IN  OTU FAR
NO.       LINK LINK LINKS  AVG.     OTU
-------------------------------------------------------------
  1   53                                  31-276  v-a
      54 0.01                             29-268  v-a
       8 0.02   54 0.021 0.007  53 0.02   37-520  v-a
       7 0.02   54 0.029 0.008   8 0.04   36-519  v-a
       5 0.02    8 0.029 0.000  53 0.04   34-516  v-a
       6 0.02    5 0.033 0.004  53 0.04   35-517  v-a
      10 0.02    5 0.027-0.006  53 0.04   54d-533 v-a
      51 0.02    5 0.025-0.002  53 0.03   14-566  v-a
       2 0.02   51 0.026 0.002  53 0.04   4-524   v-a
       9 0.02    5 0.035 0.009   7 0.05   38-523  v-a
      55 0.02   53 0.034-0.000  10 0.04   16b-194 v-a
       4 0.02    9 0.043 0.008  53 0.06   15-567  v-a
       1 0.02   55 0.032-0.011   4 0.05   1-572   v-a
      12 0.02   51 0.033 0.000  53 0.04   57-579  v-s
      14 0.02   12 0.034 0.001   6 0.04   60-604  v-s
      52 0.02    1 0.037 0.003   4 0.05   30-547  v-a
      50 0.03   51 0.045 0.008  52 0.05   12-565  v-a
      56 0.03   51 0.045 0.000  53 0.06   28-256  v-a
      13 0.03    6 0.050 0.006  54 0.07   58-590  v-s
      11 0.05   51 0.059 0.009  13 0.08   56-551  v-a
       3 0.06    1 0.089 0.030  54 0.11   9-550   v-a
      49 0.05    3 0.091 0.002  55 0.12   7-568   v-a
      15 0.07    6 0.086-0.005   3 0.12   10-556  v-a
      48 0.08   56 0.112 0.027  49 0.17   5b-548  v-a
      16 0.08    1 0.103-0.009  48 0.15   25-545  v-a

      20 0.11   15 0.133 0.030  16 0.19  200 50-471  v-o
-------------------------------------------------------------
  2   21                                  52-486  v-bc
      23 0.04                             21-499  v-bc
      19 0.05   23 0.059 0.015  21 0.07   24-488  v-bc
      20 0.07   23 0.085 0.026  19 0.11   50-471  v-o
      22 0.08   23 0.105 0.020  20 0.13   53-507  v-bc
      18 0.06   22 0.111 0.006  20 0.15   23-487  v-bc
      17 0.08   22 0.121 0.011  20 0.17   22-489  v-bc

      15 0.11   20 0.161 0.040  17 0.22  204 10-556  v-a
-------------------------------------------------------------
  3   34                                  63-631  l-m
      36 0.06                             65-654  l-a
      33 0.08   36 0.083 0.023  34 0.09   62-622  l-l
      35 0.08   33 0.101 0.017  34 0.13   64-648  l-a

      59 0.16   36 0.206 0.105  35 0.23 1200 2-112   n-a
-------------------------------------------------------------
  4   27                                  16a-573 n-a
      28 0.06                             17-576  n-a
      26 0.06   27 0.067 0.004  28 0.07   13-571  n-a
      25 0.07   28 0.078 0.011  27 0.08   8-538   n-a
      57 0.07   27 0.088 0.010  25 0.10   5a-549  n-a
      58 0.08   25 0.087-0.000  57 0.09   55-546  n-a
      24 0.09   58 0.116 0.029  25 0.14   6a-570  n-a
      29 0.09   57 0.108-0.008  24 0.14   54a-537 n-a
      32 0.09   57 0.138 0.031  24 0.19   27-531  n-a
      59 0.09   25 0.129-0.009  24 0.20   2-112   n-a
      30 0.07   59 0.150 0.021  24 0.21   59-596  n-s
      31 0.09   30 0.131-0.020  24 0.18   26-253  n-a

      36 0.16   59 0.211 0.080  27 0.23 1204 65-654  l-a
-------------------------------------------------------------

ISOLATED OTU'S (SINGLE MEMBER CLUSTERS)

CLUSTER OTU LABEL
      5  37 51-478  m-w
      6  38 40-375  m-w
      7  39 41-394  m-w
      8  40 42-396  b-w
      9  41 43-398  m-w
     10  42 44-414  p-w
     11  43 45-442  m-o
     12  44 46-443  o-o
     13  45 47-458  a-o
     14  46 48-459  m-c
     15  47 49b-463 m-c

CLUSTER MEMBERSHIP INDEX

   1   1    11   1    21   2    31   4    41   9    51
   2   1    12   1    22   2    32   4    42  10    52
   3   1    13   1    23   2    33   3    43  11    53
   4   1    14   1    24   4    34   3    44  12    54
   5   1    15   1    25   4    35   3    45  13    55
   6   1    16   1    26   4    36   3    46  14    56
   7   1    17   2    27   4    37   5    47  15    57
   8   1    18   2    28   4    38   6    48   1    58
   9   1    19   2    29   4    39   7    49   1    59
  10   1    20   2    30   4    40   8    50   1
```

```
MAPPING AIDS
------------

CENTRALITY-PERIPHERALITY ORDER
OTU'S        39  41  34  47  33  45  35  31  26  40  57  43  28  18  20  19  44  32  23  30  22  36  27  17  29
CLUSTERS      7   9   3  15   3  13   3   4   4   8   4  11   4   2   2   2  12   4   2   4   2   3   4   2   4

OTU'S        25  46  15  58  59  21  42  38   1  24  52   3   9  14  12   4  50   8   5  48   6  56  13  49  10
CLUSTERS      4  14   1   4   4   2  10   6   1   4   1   1   1   1   1   1   1   1   1   1   1   1   1   1   1

OTU'S        51   2   7  16  55  11  54  53  37
CLUSTERS      1   1   1   1   1   1   1   1   5

CLUSTER DISTANCES (*1000)
     MAIN DIAGONAL = CLUSTER RADII (I.E. 1/2 THE DISTANCE BETWEEN
          THE TWO MOST DISTANT OTUS IN THE CLUSTER)
     LOWER TRIANGLE = CENTER TO CENTER DISTANCES OF CLUSTER CIRCLES
          (I.E. RADIUS 'A' + RADIUS 'B' + DISTANCE BETWEEN NEAREST NEIGHBORS IN A AND B)
     UPPER TRIANGLE = MINIMUM INTER-CLUSTER DISCONTINUITIES
CLUSTER        1         2         3         4         5         6         7         8         9        10
   1          84       107       214       168       515       433       251       354       254       412
   2         276        84       230       217       475       422       288       357       306       421
   3         365       381        66       163       365       308       270       331       293       375
   4         357       404       332       103       413       379       315       344       345       447
   5         600       560       431       517         0       305       313       299       374       264
   6         518       506       374       483       305         0       326       259       281       326
   7         336       372       336       419       313       326         0       251       186       199
   8         439       441       397       447       299       259       251         0       276       249
   9         339       380       359       448       374       281       186       276         0       263
  10         497       506       441       550       264       326       199       249       263         0
  11         426       425       401       461       303       270       252       212       276       253
  12         485       469       355       467       257       271       297       230       285       246
  13         411       427       320       420       324       189       250       209       209       249
  14         448       488       457       502       301       279       222       251       235       192
  15         389       377       310       441       316       273       221       241       173       235

CLUSTER       11        12        13        14        15
   1         341       400       326       363       304
   2         341       384       343       404       293
   3         334       289       254       391       244
   4         358       363       317       399       337
   5         303       257       324       301       316
   6         270       271       189       279       273
   7         252       297       250       222       221
   8         212       230       209       251       241
   9         276       285       209       235       173
  10         253       246       249       192       235
  11           0       162       279       217       196
  12         162         0       234       300       170
  13         279       234         0       293       202
  14         217       300       293         0       274
  15         196       170       202       274         0
```

VIII. APPENDIX 3. TAXMAP Analysis 2.

TAXMAP analysis 2 contained 72 OTUs including a 13 OTUs representing typespecimens (holotypes, isotypes, and paratypes) along with the 59 OTUs used for TAXMAP analysis 1 (Appendix 2). A weighted analysis employed the relative information content of the attributes to calculate the relative inter-OTU distances.

Only the TAXMAP cluster analysis is listed here.

```
MAP CLUSTER ANALYSIS:-NUTTALLIANAE, 1983: 72 OTUS, ATT 1-50, weighted

(MINIMUM NUCLEUS 0.102, MAXIMUM DROP 0.0400 BOTH ARE 100% OF NORMAL)

CLUS OTU DIST OTU  AVGOF  DROP FAR DIST FLAG NAME OF OTU
TER  NOS BEST BEST  NEW    IN  OTU FAR
NO.      LINK LINK LINKS  AVG.      OTU
-------------------------------------------------------------
  1   53                                    31-276  v-a
      54 0.03                               29-268  v-a
       7 0.03  54 0.042 0.017  53 0.05      36-519  v-a
       2 0.03   7 0.045 0.003  53 0.06      4-524   v-a
      51 0.03   2 0.042-0.004  53 0.05      14-566  v-a
      10 0.03   2 0.044 0.002  53 0.06      54d-533 v-a
       5 0.03  10 0.043-0.000  53 0.06      34-516  v-a
       6 0.03   5 0.048 0.004  53 0.07      35-517  v-a
       9 0.03   5 0.055 0.007  54 0.08      38-523  v-a
       8 0.03   5 0.039-0.016   7 0.05      37-520  v-a
       1 0.03  51 0.046 0.007   7 0.06      1-572   v-a
      52 0.03   1 0.057 0.011   6 0.07      30-547  v-a
       4 0.03   9 0.067 0.011  53 0.10      15-567  v-a
      12 0.03   8 0.047-0.021  53 0.07      57-579  v-s
      14 0.03  12 0.052 0.005   4 0.07      60-604  v-s
      55 0.04  53 0.064 0.012   4 0.09      16b-194 v-a

      64 0.04  54 0.115 0.051   4 0.21 1000 S8530   type-s
-------------------------------------------------------------
  2   13                                    58-590  v-s
      56 0.05                               28-256  v-a

      52 0.05  56 0.064 0.012  13 0.07    4 30-547  v-a
       LINK TO CLUSTER  -1
-------------------------------------------------------------
  3   34                                    63-631  1-m
      36 0.06                               65-654  1-a
      33 0.07  34 0.071 0.010  36 0.07      62-622  1-1
      70 0.08  33 0.107 0.036  34 0.14      CFB225  type-g
      69 0.08  70 0.146 0.039  36 0.19      AN5481  type-e
      35 0.09  33 0.108-0.038  69 0.12      64-648  1-a

      47 0.10  70 0.180 0.071  35 0.25 1000 49b-463 m-c
-------------------------------------------------------------
  4    3                                    9-550   v-a
      50 0.06                               12-565  v-a

       7 0.06  50 0.077 0.014   3 0.09    4 36-519  v-a
       LINK TO CLUSTER  -1
-------------------------------------------------------------
  5   21                                    52-486  v-bc
      23 0.07                               21-499  v-bc
      19 0.07  23 0.087 0.021  21 0.11      24-488  v-bc
      62 0.07  23 0.082-0.006  21 0.09      AN4340  type-v

      10 0.07  62 0.113 0.031  19 0.15    4 54d-533 v-a
       LINK TO CLUSTER  -1
-------------------------------------------------------------
  6   24                                    6a-570  n-a
      58 0.07                               55-546  n-a
      25 0.08  58 0.100 0.031  24 0.12      8-538   n-a
      28 0.08  25 0.111 0.010  24 0.14      17-576  n-a
      27 0.08  28 0.092-0.018  58 0.10      16a-573 n-a
      57 0.08  27 0.098 0.006  28 0.12      5a-549  n-a
      32 0.08  57 0.118 0.020  24 0.14      27-531  n-a
      26 0.09  25 0.111-0.007  32 0.16      13-571  n-a
      59 0.09  57 0.111-0.000  24 0.15      2-112   n-a
      29 0.10  25 0.120 0.009  59 0.15      54a-537 n-a
      30 0.10  59 0.136 0.016  26 0.19      59-596  n-s
      31 0.10  25 0.120-0.016  24 0.14      26-253  n-a

      67 0.11  59 0.167 0.046  28 0.21 1000 ANSPH   type-n
-------------------------------------------------------------
```

```
           7   16                                     25-545  v-a
               49 0.08                                7-568   v-a

** NEEDED .GT. FOUND FOR NEXT OTU **

               12 0.08   49 0.355 0.273  72 0.63 1004 57-579  v-s
         -------------------------------------------------------------
           8   64                                     S8530   type-s
               66 0.09                                RS-BB   type-r

** NEEDED .GT. FOUND FOR NEXT OTU **

               54 0.09   66 0.359 0.273  72 0.63 1004 29-268  v-a
         -------------------------------------------------------------
           9   17                                     22-489  v-bc
               22 0.09                                53-507  v-bc
               18 0.10   17 0.099 0.008  22 0.10      23-487  v-bc

               65 0.10   17 0.152 0.053  18 0.23 1000 CFB67   type-ph
         -------------------------------------------------------------
          10   11                                     56-551  v-a
               48 0.10                                5b-548  v-a

** NEEDED .GT. FOUND FOR NEXT OTU **

                9 0.10   48 0.364 0.268  72 0.63 1004 38-523  v-a
         -------------------------------------------------------------
          11   15                                     10-556  v-a
               20 0.10                                50-471  v-o

               64 0.10   20 0.135 0.033  15 0.17    4 S8530   type-s
                LINK TO CLUSTER  -8
         -------------------------------------------------------------

         ISOLATED OTU'S (SINGLE MEMBER CLUSTERS)

         CLUSTER OTU LABEL
              12  37 51-478  f-w
              13  38 40-375  m-w
              14  39 41-394  m-w
              15  40 42-396  b-w
              16  41 43-398  m-w
              17  42 44-414  p-w
              18  43 45-442  m-o
              19  44 46-443  o-o
              20  45 47-458  a-o
              21  46 48-459  m-c
              22  47 49b-463 m-c
              23  60 B9634    ltype-p
              24  61 HOOK/k   type-m
              25  63 S10200   type-x
              26  65 CFB67    type-ph
              27  67 ANSPH    type-n
              28  68 B8662    type-o
              29  71 H3156    type-f
              30  72 SMH222   ctype-f

         CLUSTER MEMBERSHIP INDEX

           1   1     11  10     21   5     31   6     41  16     51   1     61  24     71  29
           2   1     12   1     22   9     32   6     42  17     52   1     62   5     72  30
           3   4     13   2     23   5     33   3     43  18     53   1     63  25
           4   1     14   1     24   6     34   3     44  19     54   1     64   8
           5   1     15  11     25   6     35   3     45  20     55   1     65  26
           6   1     16   7     26   6     36   3     46  21     56   2     66   8
           7   1     17   9     27   6     37  12     47  22     57   6     67  27
           8   1     18   9     28   6     38  13     48  10     58   6     68  28
           9   1     19   5     29   6     39  14     49   7     59   6     69   3
          10   1     20  11     30   6     40  15     50   4     60  23     70   3
```

IX. APPENDIX 4. TAXMAP Analysis 3.

TAXMAP analysis 3 contained 39 OTUs 17 OTUs from the author's collection, 8 OTUs from cytologically known herbarium specimens which were extracted for flavonoid profile analysis, and 8 type specimens. In an attempt to clarify the relationships between *V. praemorsa, bakeri* and *linguaefolia*, all specimens of *V. vallicola* and *V. nuttallii* were removed from this analysis.

A weighted analysis was performed using attributes 1 to 50. The resulting output is given in the form of a cluster analysis table. A second TAXMAP analysis was performed using only the morphological attributes, 30 to 50. The resulting output from the morphological analysis is also given as a cluster analysis table.

```
MAP CLUSTER ANALYSIS:-NUTTALLIANAE, PRAEMORSA: 39 OTUS, ATT 1-50, WEIGHTED

(MINIMUM NUCLEUS 0.180, MAXIMUM DROP 0.0318 BOTH ARE 100% OF NORMAL)

CLUS OTU DIST OTU  AVGOF  DROP FAR DIST FLAG NAME OF OTU
TER  NOS BEST BEST  NEW    IN  OTU FAR
NO.      LINK LINK LINKS  AVG.      OTU
------------------------------------------------------------
  1   2                                    63-631  l-m
      4 0.07                               65-654  l-a
      1 0.08   4 0.084 0.012   2 0.09      62-622  l-l
     37 0.09   1 0.115 0.031   2 0.15      CFB225  type-g
     36 0.09  37 0.162 0.047   4 0.20      AN5481  type-e
      3 0.10   4 0.124-0.038   2 0.15      64-648  l-a

     15 0.10  37 0.212 0.088   3 0.32 1000 49b-463 m-c
------------------------------------------------------------
  2  22                                    B8388   b-c
     28 0.09                               B8052   b-c
     25 0.10  28 0.120 0.031  22 0.14      B8377   b-c
     23 0.12  28 0.150 0.030  22 0.18      B5215   bg-c

     37 0.13  23 0.206 0.056  22 0.26 1004 CFB225  type-g
------------------------------------------------------------
  3  19                                    B8385   a-c
     29 0.10                               B11462  ltype-a
     30 0.10  29 0.126 0.031  19 0.15      B12086  ltype-a

     15 0.13  29 0.185 0.058  30 0.22 1000 49b-463 m-c
------------------------------------------------------------
  4  31                                    B7408   m-l
     35 0.10                               B8662   type-o
     32 0.11  31 0.150 0.053  35 0.19      A8317   a-o
     27 0.11  32 0.141-0.008  31 0.16      GD1640  m-n

     37 0.11  32 0.175 0.033  35 0.22    4 CFB225  type-g
      LINK TO CLUSTER  -1
------------------------------------------------------------
  5  11                                    45-442  m-o
     12 0.14                               46-443  o-o typesite

     37 0.16  11 0.159 0.022  12 0.16    4 CFB225  type-g
      LINK TO CLUSTER  -1
------------------------------------------------------------
  6  20                                    B8403   b-c
     24 0.16                               B8406   b-c

     13 0.16  20 0.199 0.042  24 0.24 1000 47-458  a-o typesite
------------------------------------------------------------
  7   6                                    40-375  m-w
     26 0.16                               D1756   m-l

      5 0.17  26 0.218 0.055   6 0.26 1000 51-478  m-w
------------------------------------------------------------
  8  16                                    B9634   ltype-p
     17 0.17                               B8059   p-c

     31 0.19  16 0.203 0.036  17 0.22 1004 B7408   m-l
------------------------------------------------------------
  9   8                                    42-396  b-w

     21 0.18                               B7354   o-c

     23 0.18  21 0.183 0.007   8 0.18    4 B5215   bg-c
      LINK TO CLUSTER  -2
------------------------------------------------------------
 10  38                                    H3156   type-f
     39 0.18                               SMH222  co-type-f
      5 0.19  39 0.197 0.020  38 0.21      51-478  m-w

     37 0.19   5 0.244 0.047  39 0.33 1004 CFB225  type-g
------------------------------------------------------------

ISOLATED OTU'S (SINGLE MEMBER CLUSTERS)

CLUSTER OTU LABEL
     11   7 41-394  m-w
     12   9 43-398  m-w
     13  10 44-414  p-w ltypesite
     14  13 47-458  a-o typesite
     15  14 48-459  m-c
     16  15 49b-463 m-c
     17  18 D1738   m-n
     18  33 B8699   t-c
     19  34 S10200  type-x

CLUSTER MEMBERSHIP INDEX

   1   1     11   5     21   9     31   4
   2   1     12   5     22   2     32   4
   3   1     13  14     23   2     33  18
   4   1     14  15     24   6     34  19
   5  10     15  16     25   2     35   4
   6   7     16   8     26   7     36   1
   7  11     17   8     27   4     37   1
   8   9     18  17     28   2     38  10
   9  12     19   3     29   3     39  10
  10  13     20   6     30   3
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MAP CLUSTER ANALYSIS:-NUTTALLIANAE, PRAEMORSA: 39 OTUS, ATT 30-50, WEIGHTED

(MINIMUM NUCLEUS 0.148, MAXIMUM DROP 0.0350 BOTH ARE 100% OF NORMAL)

CLUS OTU DIST OTU  AVGOF  DROP FAR DIST FLAG NAME OF OTU
TER  NOS BEST BEST  NEW    IN  OTU FAR
NO.      LINK LINK LINKS  AVG.     OTU
------------------------------------------------------------
  1   29                                  B11462  ltype-a
      30 0.05                             B12086  ltype-a
      19 0.08   30 0.083 0.032  29 0.09   B8385   a-c
      27 0.08   29 0.087 0.004  19 0.10   GD1640  m-n
      20 0.08   27 0.121 0.034  19 0.14   B8403   b-c
      13 0.09   27 0.131 0.010  19 0.17   47-458  a-o typesite
      32 0.10   13 0.123-0.008  29 0.14   A8317   a-o
      31 0.09   32 0.146 0.023  29 0.20   B7408   m-l
      35 0.10   31 0.180 0.034  19 0.25   B8662   type-o
      37 0.11   32 0.198 0.017  30 0.25   CFB225  type-g
       1 0.09   37 0.175-0.023  35 0.25   62-622  l-l
       4 0.08    1 0.168-0.006  35 0.22   65-654  l-a
      23 0.08    4 0.175 0.006  29 0.25   B5215   bg-c
       2 0.09    4 0.153-0.021  31 0.21   63-631  l-m
       3 0.09    4 0.183 0.030  13 0.28   64-648  l-a
      24 0.09   23 0.169-0.014  29 0.22   B8406   b-c

      22 0.09   24 0.204 0.035  29 0.27 1000 B8388   b-c
------------------------------------------------------------
  2   22                                  B8388   b-c
      25 0.06                             B8377   b-c
      28 0.06   25 0.072 0.017  22 0.08   B8052   b-c

      24 0.09   22 0.103 0.031  28 0.11    4 B8406   b-c
       LINK TO CLUSTER  -1
------------------------------------------------------------
  3    8                                  42-396  b-w
      21 0.07                             B7354   o-c

      24 0.13    8 0.134 0.062  21 0.14    4 B8406   b-c
       LINK TO CLUSTER  -1
------------------------------------------------------------
  4   16                                  B9634   ltype-p
      17 0.11                             B8059   p-c

      31 0.14   16 0.173 0.067  17 0.21 1004 B7408   m-l
------------------------------------------------------------
  5    5                                  51-478  m-w
      26 0.12                             D1756   m-l

      19 0.16   26 0.198 0.080   5 0.23 1204 B8385   a-c
------------------------------------------------------------
  6   11                                  45-442  m-o
      12 0.12                             46-443  o-o typesite

      31 0.15   11 0.191 0.070  12 0.24 1004 B7408   m-l
------------------------------------------------------------
```

```
ISOLATED OTU'S (SINGLE MEMBER CLUSTERS)

CLUSTER OTU LABEL
      7   6 40-375  m-w
      8   7 41-394  m-w
      9   9 43-398  m-w
     10  10 44-414  p-w ltypesite
     11  14 48-459  m-c
     12  15 49b-463 m-c
     13  18 D1738   m-n
     14  33 B8699   t-c
     15  34 S10200  type-x
     16  36 AN5481  type-e
     17  38 H3156   type-f
     18  39 SMH222  co-type-f

CLUSTER MEMBERSHIP INDEX

  1   1     11   6     21   3     31   1
  2   1     12   6     22   2     32   1
  3   1     13   1     23   1     33  14
  4   1     14  11     24   1     34  15
  5   5     15  12     25   2     35   1
  6   7     16   4     26   5     36  16
  7   8     17   4     27   1     37   1
  8   3     18  13     28   2     38  17
  9   9     19   1     29   1     39  18
 10  10     20   1     30   1
```

X. APPENDIX 5. Representative Specimens

Viola nuttallii

CANADA, Alberta: *D Fabijan 570* (ALTA), N bank of Bow River, Scandia, 2400′; *538* (ALTA), Cochrane Hill, Cochrane, 4000′; *571* (ALTA), N bank Old Man River, Taber, 2500′; *573* (ALTA), Etzikom Coulee 38.6 km S of Taber on hwy 36, 3100′; *576* (ALTA), Writting-On-Stone, 3000′; *537* (ALTA), Nose Hill, Calgary, 3700′; *253* (ALTA), Fabyan, 2100′; *531* (ALTA), 18 km N of Wainwright on hwy 41, 2000′; *549* (ALTA), 5.7 km E Cluny on hwy 1, 2800′; *546* (ALTA), Turner Valley, 4100′; *C Strand 18* (ALTA, DAO), 2 mi S of Pearce, Old Man River Valley; *EH Moss 1107* (ALTA, DAO, G), S of Lethbridge; *MG Dumais 6054* (ALTA), W of Wainwright at Battle River Campsite, hwy 14; *6514* (ALTA), Drumheller, W side of River; *WC McCalla 11071* (ALTA), W of MacLeod; *7355* (ALTA), Calgary; *4594* (ALTA, UBC), North Hill, Calgary; *8554* (ALTA, UBC), Riley Park, Calgary; *8557* (G, UBC), N of Riley Park, Calgary; *8576* (G), N of Riley Park, Calgary; *8601* (ALTA, UBC), N of Riley Park, Calgary; *9382* (ALTA, DAO, G, UBC), N of Riley Park, Calgary; *10313* (ALTA, UBC), N of Riley Park, Calgary; *12850* (ALTA, UBC), N of Riley Park, Calgary; *HW Pinel and C.A. Wallis V-77* (CAN), 4 mi ENE of Rosebud, 2600′; *V-12* (DAO), 1/4 mi W of Gatine, 2350′; *HJ Scoggan 16607* (CAN), Pincher Creek; *ME Moodie 821* (G, MO, US), Black Birch Coulee, Rosedale, 2200-2500′.

Saskatchewan: *D Fabijan 596* (ALTA), 3.5 km S and 2.3 km W of Wood Mountain Park; *B deVries 468* (DAO), E of Lebret, Qu'Appellee Valley; *B Boivin 13450* (DAO), R. Tete-a-la-Biche, 3 mi S of Carievale; *RJ Ledingham and R.C. Russell S4910* (DAO), Moose Jaw; *S4916* (DAO), Elbow; Sask. R. Valley; *S5036* (DAO), Outlook, towards river; *B.J. Sallans and R.C. Russell* (DAO), Fort Qu'Appelle; *L.T. Carmichael R-14* (DAO), Qu'Appelle Valley; *R.C. Russell and W.P. Fraser* (DAO), Sutherland; *B.J. Sallans and R.C. Russell* (DAO), Buffalo Pound Lk., Chamberlain; *N.A. Skoglund 26* (DAO,CAN,G), 35 km SE of Kyle; *R.C. Russell S4736* (DAO), Katepwe, Qu'Appelle Valley; *2828* (DAO), Katepwa; *G.F. Ledingham 49-13* (DAO), N of Wascana Pond, Regina; *J.H. Hudson 1937* (DAO), Mortlach, Moose Jaw district; *G.J. Jones 634* (DAO), Lake Marguerite, 12 mi S of

Indian Head; *W.H. Cameron* (DAO), Sutherland: *W.P. Fraser* (DAO), Saskatoon.

Manitoba: *B. Boivin 13427* (DAO), 1 mi SE of Goodlands; *G. Stevenson 431* (DAO), Brandon; *H.H. Marshall 34* (DAO), Brandon: *T.J.W. Burgess* (DAO), first crossing, Souris River; *G.M. Dawson 198* (DAO), E crossing, Souris River; *H.J. Scoggan 7183* (CAN), Souris River, 8 mi N of Minto; *G.A. Stevenson 766* (CAN), Brandon; *J. Macoun 12446* (CAN), entrance to Exp. Farm, Brandon; *R.D. Bird* (ALTA), Little Saskatchewan River, Brandon; *B Boivin 13429* (DAO,G), W of Dalny, Souris River.

UNITED STATES: Montana: *WW Jones* (RM,DS,G,UC,US), Gallatin Co., Bozeman; *EJ Moore* (RM,DS,US,G), Gallatin Co., Bozeman; *JW Blankinship 552* (RM,PH,DS,US,UC,MO), Bozeman; (UC,G), Broadwater Co.,Lombard; *Hoag and Mayer 1* (RM), Decker Quadrangle, Big Horn Co., 3430-3800'; *WE Booth* (RM), 2 mi N of Ryegate, Golden Valley Co.; *LC Ellison 3301* (RM), Custer Co., 2800'; *AW Collotzi and G Davidse 647* (CAS,DS,PH,G,US,WS), S of Billings airport, Yellowstone Co.; *HR Flint 46* (RM), Long Pines Div., Custer Forest, 3800'; *TN Traphagen* (PH), Deer Lodge; *HT and JM Rogers 1334* (WS), Lambert, Richland Co.; *PH Hawkins* (UC), Absarokee; *ED Wooton* (US), Vicinity of Helena, Clark Co; *J Roseneau* (DAO), coulee near Giant Sprints Rocky area, Great Falls, Cascade Co; *E. Starz* (MO), Helena.

North Dakota: *J. Lunnel* (DS,RM,PH,MO,US), Leeds, Benson Co; (G), Butte, Benson Co; *W.F. Bergman 1370* (DS), Enderlin; *E.T. Wherry* (PH), Stark Co., 3.5 mi W Dickinson; *C.L. and M.W. Porter 10,368a* (RM), Billings Co., Medora and T. Roosevelt Nat. Mem. Park, 2500'; *W.F. Bergman 1434* (PH), Kathyrn; *L.F. Lautenschlager 206* (CAN), Ward Co.; *J.T. Sarvis 39* (US), Mandan, Morton Co.; *O.A. Stevens 2587* (CAN, UC, US), Morton Co., Glen Ullin; (US), Steele; *W. Harken* (US), prairies; *W.N. Suksdorf (WS), Sedalia;* *J.F. Brenkle* (MO), Kulm; *J.F. Bergman 1370* (MO), Enderlin; *R.P. Williams 880* (MO), Shell Butte, 13.5 mi S, 3 mi W of Napolean.

South Dakota: *W.F. Forwood 38* (US,CAN), Meade Co., Black Hills near Ft. Meade; *F.L. Bennett 72* (MO), N Black Hills, N Spearfish; *D. Griffiths (GH, US), Brown Co., Aberdeen; J.J. Thornber* (GH, UC, MO), Iroquis; *J.F. Brenckle 41-06* (GH, MO), Northville; *L.A. Hanna 141* (MO), Quashnick farm, Artas; *W. Larwence* (WS), Lake Kampesky; *W.H. Over 2097* (US), Bear Creek, Washabaugh Co.; *P.A. Rydberg 550* (GH), Hot Springs, 3500'; *J. Murdock 4060* (GH), Rocky Ridge, Bald Hills, 5200'; *E.T. Wherry* (PH), Fall

R. Co., 8 mi E Smithwick, *C.A. Barr 1059* (PH), 8 mi SE Smithwick; *R.R. Krebs 62* (RM), Jackson Co., Buffalo Gap Nat. Grassland, 2500'; *H.E. Hayword 655* (RM), Badlands Cedar Canyon; *985* (RM), Boulder Cr. Park, 6 mi E Deadwood; *938* (RM), Kermosa; *H. Lee 532* (RM), Rapid City; *G.M. Dodge 21* (RM), Custer Forest, Eagle's Nest Ranger Station; *L.W. Shevling 34.5* (RM), Custer Forest.

Nebraska: *E.S. Nixon 4* (RM), Sheridan Co., 13 mi N Hay Springs; *J.M. Bates* (RM), Valentine; *R.L. McGregor 19869* (DS), Garden Co., 4 mi S Lewellen; *F.V. Hagen* (MO), Willow Creek; *W.L. Todstead 12* (GH), Holt Co., Niobrara R. valley N of O'Neill; *41* (GH), a mi W of Ohadron; *S. Stephens 38080* (GH), Deuel Co., 2 mi N, .5 W Big Springs; *S. Stephens and R. Brooks 11587* (GH), Scotts Bluff Co., 2 mi W of Gering; *S. Stephens 38062* (GH), Keith Co., 13 mi W Lamoyne.

Kansas: *L. Watson* (GH), Ellis.

Wyoming: *Hayden* (GH), Cheyenne, Wyoming Terr.; *M. Cary 315* (US), W of Isley; *L. Williams 2185* (UC, US, WS), 1 mi E of Laramie, Albany Co.; *C.L. and M.W. Porter 7747* (DAO, UC, CAS), near Rock River, Albany Co.; *7734* (RM), Sybille Cr., Platte Co.; *H. Symons 51* (CAN), near Laramie; *A. Brebner 5* (CAN), N or Laramie; *K. Roach* (CAN), Laramie plains; *A. Nelson 6826* (CAN, PH, DS, CAS, UC, MO, RM, US, WS, GH), Laramie hills, Albany Co.; *226* (GH, RM), Laramie hills; *6956* (GH, MO, US, RM), Sand Cr., Albany Co.; *209* (UC), Laramie; *37* (GH, US, PH, RM), Laramie hills; *1229* (RM), Laramie; *M. Ownbey 544a* (UC, WS, RM, DS), 5 mi NW Hulett, Crook Co., 4300'; *544b* (UC, RM, DS), 7 mi NW Hulett, Crook Co., 4500'; *583* (MO, UC, WS, DS), 8 mi NW Hulett, Crook Co., 4500'; *F. Tweedy 4627* (US), Fort Steele, Carbon Co.; *A.A. Beetle 10359* (UC), Boulton Creek Expt. Area, Natrona Co.; *J.F. Macbride 2271* (RM), Albany Co., Centennial; *B.C. Buffum* (RM), Laramie hills; *W.G. Solhiem 418* (RM), Laramie hills; *B.E. and L. Barsum 1206* (RM), Albany Co., Sheep Mtn, #11 1.9 mi SW of #130, 7800'; *J. Finzell 171* (RM), Laramie Co., 7 mi W Cheyenne, 6000'; *F.B. Current 242* (RM), Laramie Co., 5 mi NW Cheyenne, 6100'; *A. Nelson* (RM), Moorcroft;*9614* (RM), Bird's Eye; *B. Dickson 273* (RM), Sheridan Co., Big Horn For., 4500'; *D.G. Reardon and L.M. Mayor 136* (RM), Sheridan Co., Big Horn coal site; *B.E. Nelson 1586* (RM), Niobrara Co., 22.5 mi NW Lusk, 5200'; *1535* (RM), Platte Co., 21 mi N Guernsey, 5350'; *C.L. Turner 63* (RM), Converse Co., W of Douglas; *E.B. Snell* (DS), Buffalo; *B. Luce 2* (RM), Goshen Co., Table Mtn Wildlife Unit; *D.L. Martin*

1520 (RM), Hot Springs Co., Eagles Nest Ranch, 6770'; *1145* (RM), Kirby, Little Sand Draw oilfield; *G.R. Kleeberger* (CAS), Wyo.; *J.F. Macbride 2720* (RM), Rock River, 7000'; *Cox, Dunn, F. Leak 1764* (RM), Goshen Co., 1.5 mi N Lingle.

Arizona: *L.M. Newlon 783* (JEPS), Yarapai Co., Ash Fork, 20 mi SW.; *H.H. Rusby* (US), Ash Fork.

Colorado: *E.L. Johnston 8746* (US), Lyons, Boulder Co.; *837* (GH), Trinidad; *886* (GH), Morley; *R.F. Daubenmire* (WS),Boulder Co., Redrocks, Boulder; *Ramely and Richards 15950* (UC, WS, DS, PH, CAS, RM), Black shales N of Boulder; *C.T. Robbins 355* (UC), Flagstaff Mtn, ca .5 mi W of Boulder; *514* (UC), Las Aminas Co., E of Delagua; *K.M. and M.C. Wiegand 1485* (GH), Crouse, Larimer Co.; *C.S. Crandell 469* (US), Howes Gulch; (US), Dixon Canon; (US, CAN, UC, GH, RM, DS), Horsetooth Gulch; (GH, CAN, US, RM), Bluffs N of La Porte; *J.H. Cowan* (US, RM), Horsetooth Gulch; *59*, (MO), Fort Collins; *J.H. Ehlers 6914* (GH), Lookout Mtn, Golden, Jefferson Co., 5600'; *7961* (GH), Golden; *6788* (GH), Walsenberg, Huerfana Co.; *6774* (GH), Morley, Las Animas Co.; *L.J. Dorr 209* (MO), Golden, Jefferson Co.: *I.W. Clokey 4209* (WS, US, PH, CAS, RM), Clear Creek, Jefferson Co.; *2676* (CAN, UC), Prairie, Denver; *2767* (PH, RM), Denver; *R. Duthie and I.W. Clokey 3819* (CAS, DS, CAN, DAO, GH, PH, MO, US, UC, WS, RM), Wolhurst, Douglas Co.; *J. Wolf 76(56)* (US), Denver; *M. Lamm 19393* (CAN, RM, PH), Ramah, El Paso Co.; *W.W. Eggleston 20171* (GH, RM), Branson, Las Animas Co.; *C.M. Rogers 5656* (US), W end Mesa de Maya; *R.C. Rollins 2061* (GH, UC, DS), Pueblo Co., 11 mi S of Pueblo, 4700'; *A. Nelson 11526a* (UC, RM), Walsenburg-Pueblo; *R.A. Rydberg and F.K. Vreeland 5883* (CAN, WS), Cuchara valley, near La Veta, 2100m; *5884* (RM), near Denver; *T.J. Bandergee* (GH), Canon City, 5400'; *42* (MO, UC, PH), Canon City, Colorada Terr.; *H.L. Zobel and C.W. Penland 1205* (PH, CAS), Deer Creek Canyon, Canyon City Road, 5800'; *J.A. Ewan 12985* (CAS), Las Animas Co., Berwind Canyon; *11165* (CAS), Boulder Co., Gregory Creek above Boulder; *R.K. Gierisch 1351* (RM), Clear Creek Co., Rio Grande Forest; *B.H. Smith 7* (PH), Pagosa Springs; *K.E. Phelps* (CAS), Broomfield; *R.K. Gierisch 869* (RM), 9 mi N Denver; *C.S. Williamson* (PH), Denver Co.; *J.M. Coulter* (PH), Denver; *E.L. Berthand* (RM), Golden, Jefferson Co.; *A. Eastwood 5392* (CAS), Clear Creek Valley, 1587m; *G.E. Osterhaut 680* (RM), 2-3 mi E Spring Canon; *5238* (RM), Gulch E of Horsetooth Mtn; (RM), Weld Co., New Windsor; (RM), Dale Creek; *B. Hammel 208* (RM), 3 mi N Livermore hwy 287, 5600'; *G.S. Dodds 1979* (RM), Valley-Fossil Creek; *C.F. Baker 801* (RM, US, MO), Larimer Co.; *W.S. Cooper 74* (RM), Estes Park, Forks of Big Thompson, 6000'; *F. Tweedy 5565* (RM), near Boulder; *F. Ramaley 1530* (RM), near Boulder; *40* (RM), near Boulder; *A.G. Vestal 343* (DS), Boulder; *A.L. Bacon* (PH), Greeley; *M.E. Newlin* (PH), Colorado; *W.A. Henry 165* (PH, MO), Boulder Canon; *E. Hall and J.P. Harbour 55* (US, GH), Lat 41°; *H.L. Shantz 1359* (US), Akron; *E. Palmer* (US), Colo. Terr.; *H.C. Hanson c482* (MO), Boulder; *F. Nislizenus 1169* (MO), Denver.

Viola vallicola A. Nels.

CANADA, British Columbia: *H. Groh* (DAO), Bridesville; (DAO), Summerland; *J. Bostock* (DAO), Keremeos; (DAO), Summerland; *Hitchins 1-003* (UBC), Hat Creek, 3300'; *Beamish, Luetiens, Krause 680015* (CAN), Old Cawston Road, Oliver, 2800'; *Beamish and Vrugthon 60298* (UBC), Oliver; *W.B. Johnston* (UBC), Newgate; *E. Jacobs 56* (UBC), Midway; *Spreadborough 70,877* (CAN), Midway; *D. Morrison* (UBC), Flatiron Mtn; *D.L. Krause* (UBC), 10 mi SW Princeton; *J. Grant* (UBC), Vernon, 1200'; *E.W. Warren* (UBC), Vernon; *E. Wilson 5274* (UBC), Armstrong, Okanogan; *J.W. Eastman* (UBC), Penticton; *J.A. Calder and D.B.O. Saville 8125* (DAO), 8 mi SW Okanogan Falls, 3800'; *Calder, J.A. Parmelee and R.L. Taylor 16793* (DAO), 14 mi N Merritt; *167474* (DAO, UC, US), Stump Lake 25 mi NE Merritt; *W.C. McCalla 6874* (UBC, ALTA), Cranbrook; *E. Wilson 15* (WS), Armstrong; *J.R. Anderson 69* (WS), Grand Forks; *T.T. McCabe 1975* (UC), 20.5 mi E Kamloops; *1978* (UC), 10.2 mi from Pritchard to Princeton; *5955* (UC), Anarchist Mtn, Osoyoos.
Alberta: *W.C. McCalla 8667* (ALTA), N of Calgary; *11353* (ALTA), Nose Hill, Calgary; *11382* (ALTA), Nose Hill, Calgary; *10383* (ALTA, GH), Nose Hille, Calgary; *8600* (ALTA, CAN), Normal School, Calgary; *3642* (ALTA), Normal School, Calgary; *8573* (ALTA, CAN, DAO), NW of Calgary; *8587* (ALTA, DAO), NW of Calgary; *8587* (ALTA, GH), NW of Calgary; *8567* (ALTA, DAO), King George School, Calgary; *8572* (ALTA), King George School, Calgary; *12210* (ALTA, GH), Highwood River, 6 mi W Longview; *6817* (ALTA, DAO), between Pincher Creek and Cowley; *9386* (ALTA, GH), east of Cowley; *9617* (GH), Nose Hill, Calgary; *J. Macoun 18201* (CAN), Calgary; *V.C. Brink* (UBC), Scotfield, SE of Hanna;

D. Hancock 33 (ALTA), 3 mi S of Aden; *E.H. Moss 5075* (ALTA), near DeWinton, S of Okotoks; *9777* (ALTA, CAN, DAO), near Elkwater, Cypress Hills; *McCormack* (ALTA), Cypress Hills; *Lewis and Kulyk 77-5* (CAN), Cypress Hills Prov. Park; *Spreadborough 3095* (CAN), Medicine Hat; *H.E. Groh 64* (ALTA), E of Lethbridge; *Dawson 246* (CAN), N of Milk River; *J.J. Sexsmith 513* (DAO), Brocket; *F.J. Jenkins 102703* (CAN), Porcupine Hills; *J.G. Packer 5064* (ALTA), Maskinonge Lake, Waterton; *R.C. Russell S5190* (DAO), Elkwater; *R. Kuchar 2366* (ALTA), Waterton, Blakiston Cr., 4300'; *J. Kuijt 9* (CAN), Waterton, Bellevue Ridge.

Saskatchewan: *R.J. Ledingham and R.C. Russell 4934* (DAO), Cypress Hills, 20 mi S of Maple Creek; *S4920* (DAO), Keeler; *R.C. Russell and B.J. Sallons* (DAO), Duval, Last Mountain; *B. Rawlinson and G. Ledingham 49-162* (DAO), Pilot Butte, 10 mi E of Regina; *B. Boivin 13466* (DAO), R. Plumee, 7 mi S of Carnduff; *L. Jenkins* (DAO), Hoosier; *J.H. Hudson 1933* (DAO), Trewdale; *C. Frankton 1131* (DAO), 8 mi S, 1 mi W of Saskatoon near Beaver Creek; *R.C. Russell S4129* (DAO), Aylesbury, valley of Arm River; *D.S.E. Clark* (DAO), Cypress Hills; *R.C. Russell* (DAO), Saskatoon; *W.P. Fraser* (ALTA, DAO), Saskatoon; *J. Looman* (DAO), Mortlach; *G.H. Argus 3811* (DAO, GH), nwy 5 E Borden on N Saskatchewan R.; *A.W. Anderson* (CAN), Pike Lake; *G.H. Turner 5597* (ALTA), S of Karen; *J.M. Macoun 3095* (US), Cypress Hills, NWT; (US, GH), Crane Lake, Assa.; *I.W. Clokey* (UC), Sefton.

Manitoba: *B. Boivin 13429* (ALTA), l'ouest de Dalney, Riviere Souris.

UNITED STATES, Montana: *J. Grove* (DAO), 8 mi N of Kalispel; *J.W. Blankinship 8* (US); (UC, RM), Bozeman; *77* (CAN, DS, PH, MO), Bozeman; *78* (CAN, PH, US), Bozeman; (GH), Custer; *E.O. Wooton* (US), vicinity of Helena; *F.H. Rose 27* (WS), between Drummond and Garrison; *40* (GH, MO, PH, DS, CAS, UC, WS), Miller Creel valley near Missoula; *55* (DAO, WS), Missoula Co, Send Higgins ave Paddy Cr, Missoula; *C.H. Moore* (MO), near Butte, 5500-6000'; *R.A. Coster 2* (RM), Beartooth Forest, 6000'; *C.H. Hurst 285* (RM), Absaroka Forest, 6300'; P.F. Stickney 426 (RM), Missoula Co, Mt Jumbo, Lolo Forest, .25 mi E Missoula, 3500'; *J.E. Kirkwood 1363* (GH, PH, UC), Sentinal MT, S of U, Missoula, 3300'; *P. Cantou* (CAS), Rattlesnake Cr, Bonner; *Dietter and Barkley 2347* (RM, UC, MO), N slope Mt, Sentinel, 3300'; *B.B. Irwin 99* (RM), plains about Missoula; *G.E. Osterhaut 3233a* (RM), Grand Co, Sulphur springs; *H.N. Wheeler 595* (RM), Saprinerd; *A. Nelson 5438* (GH, UC, WS, MO, DS, RM), Madison Co, 10 mi E of Monida;(RM), La Plata Mtns; *C.W. Griffins* (RM), Hodge, Wilbur Co; *W.W. Eggleston* (RM), Grey cliff, Sweet Grass Co, 1190m; *W.E. Booth 8551* (RM), Big Timber, Sweet Grass Co; *56202* (DAO), Conrad, Pondera Co, 15 mi S; *A. Roemer 718* (RM), Park Co, Absaroka Forest, Eagle Creek, 5000'; *W.W. Jones* (CAS, US, WS), Bozeman; *J. Williams 3* (DS), Bozeman, near flour mill; *E.J. Moore* (DS, GH, US), Bozeman; *B.J. Jones* (DS, MO, US), Bozeman; (GH, US), Sedan; *E.P. White 581* (RM), Gallatin Forest, 6500'; *R.L. Grabhover 7* (RM), Jefferson Forest, Carrless Creek, 6500'; *E.J. Woolfolk W-12* (RM), Custer Co, US range livestock expt stat, 2400'; *W.M. Laybourn D8-6* (RM), Jefferson Forest; *E.W. Hartwell 324* (RM), Custer Forest, La Porte School sect.; *529* (RM), Custer Forest, White tail R. St. Powder R. Co, 3600'; *323* (RM), White tail Ranger Station; *A.W. Vogelsang D2-16* (RM), Beaverhead Forest; *H.H. Hendron D4-85* (RM), Lewis and Clark Forest, 4500'; *J.E. Schmartz JES-311* (RM), Powell Co, Deerlodge, 6450'; *J.H. Thomas 2623* (RM), Mt Helen, Helena; *H.D. Cook* (GH), Bozeman; *J. Kinney* (US), Bozeman; *J.S. Flaherty* (US), Bozeman; *C.H. Draper* (US), NW of Redlodge, Carbon Co; *G. Davidse and A.W. Collotzi 644* (GH, UC, US), 10 mi E Bozeman; *W.N. Suksdorf 256* (WS), Jackrabbit Gulch, 10 mi Wilsall; (WS), Bozeman; *Nawrocki and Neff 8* (UC), Missoula River; *W.E. Booth 56269* (DAO), 12 mi S Choteau, Teton Co; *56202* (DAO), 15 mi S Conrad, Pondera Co; *A. Naasz 1055* (DAO), Watkins, Prairie Co; *J.M. Gilbert and R.M. Taylor 10927* (DAO), Lower St Mary Lake, 7 mi N of St Mary, Glacier Co; *E.W. Scheuber* (US), Yellowstone Park; (UC), Livingston; *R.S. Williams 116* (US), Columbia Falls; *P.A. Rydberg and E.A. Bessey 4541* (US), Briger Mtns, 7000'.

North Dakota: *J. Murdock 3507* (GH), Spearfish Red Beds at mouth of Beaver Cr, 3700'; *W.P. Taylor 351* (WS), Washburn; *J. Lunnell* (US, UC, GH, DS, RM), Butte, Benson Co; (PH, MO), Minot; (PH), Leeds; *R.P. Williams 866* (MO), Emmons Co, 4 mi W of Temil; *W.C. Whitman* (US), Stark Co, Dickson; *J.F. Bergman 1495* (GH), Church's Ferry; *O.A. Stevens 131* (DAO, UC, RM), Berlin; *2388* (US), Kenmare, Ward Co; *2579* (UC, US), Dunseith, Rolette Co; *Fieldstad* (RM), Valley City.

South Dakota: *W.H. Forwood 39* (US), Black Hills, Ft. Meade; *W.P. Carr 9* (CAN, GH, MO, US), Newell; *S.S. Visher 559* (RM), Date, Perkins Co; *A.C. McIntosh 62* (RM), Tilford-Elk Creek, Peidmont; *P.L. Ginter 36* (RM), Harney Forest, head of Wolf Canon, 6250';

E.T. Wherry (PH), Custer Co, 3 mi S of Custer St. Park; *C.A. Barr 1063* (PH), Custer C., 11 mi NW of Custer in Black Hills; *1064* (PH), 11 mi NW of Custer; *1061* (PH), 3 mi W Smithwick; *1060* (PH), 3 mi W Smithwick; *1062* (PH), cultivated, originally from S Custer Co 18 mi N Hot Springs; *J.F. Brenckle* (CAS), Northville; *E. Bondy 376* (CAS), Ellis Co, near Cean Hill N of Hays.

Colorado: *H.D. Ripley 5475* (CAS), 25 mi N Loma, W Garfield Creek, 6000'; *G.R. Hall 348* (RM), Whte River Forest; *R.W. Pohl 1949* (PH), Grand Co, S of Sulphur Springs; *C.R. Towne T-85* (RM), Oray Co, Uncompahgre, 8500'; *G. Van Buren GVB-22* (RM), Routte Co, Yampa, White R Forest, 7800'; *E.H. Graham 9113* (DS), Moffati, near Price Creek, 6400'; *9129* (DS), Moffat, 10 mi W Youghall, 7700'; *G.E. Osterhaut 2617* (RM), between Mecker and Craig; *2555a* (RM), Minturn, Eagle Co; *2555* (RM), Minturn, Eagle Co; *C.F. Baker 67* (UC, MO, WS), Gunnison Water Shed, Camarron, 6900'; *I. Tidestrom 3420* (US), Mt Caribou; *C.S. Crandell 469* (US), Howes Gulch; *L. Crawford* (GH, US), Routt Co, Steamboat Springs.

Wyoming: *A. Nelson 7038* (RM, GH), Sand Creek, Albany Co; *4375* (GH, MO), Plambago Canon; *8235* (GH, MO, UC, US, DS, RM), Tie City; *8943* (GH, UC, US, PH, CAS), Laramie Hills; *19* (CAN, GH, MO, UC, US), Head of Pole Creek; *1215* (US, RM), head of Pole Creek; *43* (GH, US), head of Pole Creek; (RM), Moorcroft;*9612* (RM), Birds Eye; *4525* (US), Evanston; *K. Roach* (CAN), Tie City; *C.L. Porter 3050* (GH, UC, US, DS, RM), Fox Creek, Albany Co; *8567* (DS, RM, GH, DAO, UC), Natrono Co, Casper Mtn, 8000'; *4886* (RM, DAO), N Platt R valley near Big Creek; *F.X. Jozwik 64* (GH, RM), Casper Mtn, Garden Creek Falls, 6400'; *N.H. and P.K. Holmgren 5017* (US), Lincoln Co, Twin Creek valley, old US hwy 30 N, 6750'; *L.M. Shultz 2537* (MO), Lincoln Co, Salt Creel Pass, Bridger Nat Forest; *M. Ownbey 553* (US, WS, MO, RM, DS), 7 mi NW Hulett, 4500'; *R.C. Rollins 568* (WS), Johnson Co, Upper Johnson Creek, 4000'; *S. Stephens 39523* (UC), Weston Co, 10.5 mi NE Four Corners; *R. Adams* (US), Upper Falls of the Yellowstone; *E.A. Mearns 989* (US), Mommoth Hot Springs; *F. Tweedy 408* (US), Mommoth Hot Springs; *F.H. Burgleshaus* (US), Mommoth Hot Springs; *M.S. Baker* (ALTA), Yellowstone Nat Park; *B. Maguire 13596* (GH), Yellowstone Park, 10 mi N Yellowstone Falls; *D. Mason* (US), Ft. McKinney; *E. Stevenson 73* (US), Uinta Co, La Barge; *I.J. Worthley 137* (US), Big Horn Co.; *OMurie 257* (US), Jackson; *F.V. Hayden* (MO), Wind Rain valley, 5000-8000'; *C.W.T. Penland*

1827 (CAS), Summitt Co, 8500'; *C.R. Clark 10* (RM), Albany Co, 6900'; *J.F. Macvride 2720a* (RM), Rock River, 7000'; *G.I. Sellon 72* (RM), hills E of Laramie; *B.E. and L. Nelson 398* (RM), Woods landing, S of Sheep Mt, 7800'; *1554* (RM), 20.5 mi WNW Lusk, 12.5 mi WNW Manville, 5200'; *R. O'Brien 1029* (RM), 21 mi SW Wheatland; *J. Wetherell 19* (DS), Pole Mt region, Happy Jack Rec Area, 8300'; *13* (DS, RM), Laramie Co, Laramie range, Happy Jack Road, 7500'; *W.J. Cockran C-150* (RM), Bighorn Co, Shell Ranger Station, 7750'; *R.L. Hartman 2946* (RM), Carbon Co, 2 mi NNW Elk Mtn to Hanna, 7400'; *R.B. Current 288* (RM), Shirley Basin 35 mi SSE Casper, 7110'; *309* (RM), Pathfinder Mtn, 34 mi SSE Casper, 7110'; *R. Schreibeis and K. Roman 667* (RM), Fremont Co, Gas Hills NE Jeffery City; *Olson, Gerhart, Rizer, Jones 17* (RM), above Goose Lake, 11.5 mi NE Dubois, 7280'; *H.G. Fisser 368* (RM), SW Thermopolis, Wind R Canyon, 5800'; *R.M. Hurd 329* (RM), Bighorn For, road to Cull Watt Park, 7200'; *O.C. Harrison 123* (RM), Star valley, Greys R., Afton area, 6000'; *R.E. Pfadt 156* (RM), Niobrara Co, Manville; *E&D Pearson 57* (RM), on Little Rocky, Clark's Fork valley, 5000'; *A Aldrich 26* (RM), Sweetwater Co, NE Cedar Mt, 8120'; *V Willits 12(406)* (RM), Little Goose Valley; *406* (RM), Hanna Creek; *529* (RM), Hanna Creek; *R Lichvar 92* (RM), Teton Co, Gros Ventre River; *RH Ohl 24* (RM), Teton For, Buffalo Ranger Station, 6500'; *PW Danwiddie 14* (RM), E Fork River near Boulder, 7000'; *JA Moore, LM Mayer, GG Reardon 506>* (RM), Uinta Co, 6880-7140'; *BL Coulter 67* (RM), Teton Forest, Horsetail Ranger Station, 7300'; *LA Well 66* (RM), between Fish and middle Piney Creek, 7500'; *EJ Williams F-17* (RM), Medicine Bow For, E Beaver Cr, 9000'; *FE McGrew 175* (RM), Shoshone For, 6000'.

Utah: *BF Harrison 7237* (MO), Toole Co, 10 mi S of Lolgreen; *8712* (DS), Wasatch Co, near East Portal, 8000'; *8313* (DS), Deer Cr Canyon, Utah Co, 7000'; *ME Jones (DS, CAS, PH, UC, US, MO), Echo; HM Christensen C-28* (RM), Sevier Co, Fish Lake For, Mud Flat, 7500'; *WB Miller 2-24* (RM), Fish Lake For, Birch Cr Canyon, 7500'; *EH Graham 8815* (DS, GH, US), Uintah Co, above Dry Fork, 6700'; *8084* (DS, MO), Diamond Mt plateau, 6500'; *8016* (DS, GH, UC), Duschne Co, Uinta R, 7200'; *8096* (DS, MO), Daggett Co, Grouse (summitt) Cr Canyon, 7000'; *9257* (MO), Wasatch Co, Horse Cr SW of Strawberry Resevoir, 7700'; *G Davidse 343* (DAO, GH, UC, CAS, DS), Cache Co, 11 mi up Hyrum Canyon, 5400'; *1018* (PH), Wellsville Canyon, 5000'; *356* (GH, UC, US), 1 mi up left Fork Blacksmith Fork Canyon, 5700'; *364* (UC, US), .5 mi W Herd Hollow, 6000'; *375* (UC), 20.5 mi up Logan Canyon near Twin Cr Rd, 6200'; *417* (UC), 3 mi N of Hardware Ranch, 6000'; *1045* (US), Rich Co, 1 mi W Garden City, 6100'; *993* (UC), Box Elder Co, .5 mi E Deweyville, 4600'; *1011* (UC, US), Raft R Mtns, Sawtooth Natl For, 6200'; *316* (UC, US), Juab Co, Mt Nebo, 2 mi SE Mona; *330* (GH, UC), Utah Co, 3 mi SE Thistle, Manti-LaSat Nat For, 5400'; *WC Muenscher, B Maguire 2384* (RM, GH, UC), 1 mi W Smithfield Sugar Factory; *2383* (UC), Spring Hollow, Logan Canyon; *B Maguire 3576* (UC, RM, DS, PH), 3 mi E Logan; *3566* (MO, UC, RM), Boy Scout Camp, Logan Canyon, 5400'; *CP Smith 2370* (DS, RM), Lewiston, Webster Spur; *E Tucker 1171* (DS), Boy Scout Camp, Logan Canyon; *1136* (DS), hwy at Preston Camp, Logan Canyon; *R Foster 7817* (RM), Duschne Co, Uinta River, 7200'; *L Williams 559* (CAS, GH, MO), Daggett Co, Summitt Springs Ranger Station, 9000'; *RT Best 18* (DS), Elko Co, Boy Scout Camp, 7200'; *AR Standing 112* (RM), Uinta For, Lower Pole Canyon, 6700'; *AE Aldous 3847* (RM), Manti Nat For, 6700'; *C DeMoisy 9* (RM), Ashley Forest, Whiterocks, 6000'; *M Burke 3567* (UC), Cache Co, Green Canyon, 5400'; *AH Holmgren, RJ Shaw 7642* (US), San Pete Co, Manti Nat For; *WC Clos 11* (US), Wasatch Mtns, Ephraim Canon, 2100m; *I Tidestrom 1019* (US), Wasatch Mts, Ephraim Canyon, 7500'.

Nevada: *HL Mason 4713* (UC), Cave Cr Post Office, Ruby Valley; *4760* (DS, GH, UC), 1 mi SW Cave Cr Post Office; *AH Holmgren 812* (UC), Elko Co, Mt S of Railroad Canyon, *986* (UC), 18 mi NE San Jacinto; *G Davidse and JL Gentry 1503* (GH, UC, WS, DS, RM, PH, DAO, MO), Pequop Mts, 5 mi W of hwy 40 and 30, 6790'; *1099* (UC, US), Humbolt Co, NE Winnemucca, 6100'; *1639* (GH, UC, US), Humbolt Nat For, 6100'; *H Engelmann 122* (MO),W slope Seftor valley, 6600'; *AE Hitchcock 891* (US), Kingston canyon and Birch Cr, 2250-2800m; *FS Goodner and WH Henning 131* (UC), Lander Co, Birch cr S of Austin; *P Train 2757* (UC), Kingston Co, E Toiyabe Range, 5500'; *GE Moore M-311* (RM), White Pine Co, Nevada For, 8000'; *M-610* (RM), New For, Rock Sprint near Hamilton, 8000'; *C McMillan and KH Knight 59* (RM), 40 mi E Ely; *MA King* (CAS), Warm Springs, White Pine Co; *JH and YM Robertson 49* (CAS, RM), Elko Co, Humbolt Nat For, Clover Cr, 5900'; *SL Glowenke 11023* (PH), Lincoln Co, Spring valley 5 mi W Ursine; *JM & MAR Linsdale 38M* (CAS), Kingston Cr, Landon Co, 7500'; *893* (CAS), Nye Co, S Twin River, 7300'; *HD Ripley & RC Barneby 6020* (CAS), N Wachre Co, 7 mi SW Vyr, 6000'.

Arizona: *HH Rusby 523* (UC, MO), Ash Fork, AT.

Kansas: *E Bondy 515* (MO, US), Hays.

Washington: *ZP Tammer 8* (WS), Lincoln Co, Creston Site Inventory; *TH Scheffer* (WS), Grant Co, E side upper Grand Coulee; *RG Jeffery* (WS), Adams Co, 2 mi N of Macall; *GR Vasey* (US), Washington Territory; *V Batie* (WS, RM), Okanogan Co, Twisp; *V Duthie* (DS), Spokane Co, near Spangle; *Sandberg & Leiberg 47* (DS, CAS, US, MO, UC, GH), Hangman Cr, Spokane Co; *HT Rogers 350* (CAS, DS, PH, ALTA, US, UC, MO, WS, GH), Ferry Co, Columbia River, 10 mi N Hunters Ferry, 1290'; *CS Williamson* (PH), Almota; *CV Piper 1715* (RM, GH, US, WS), Pullman; *A Radigan 91* (RM), Colville For, 3300'; *ME Jones* (DS), Davenport; *WN Suksdorf 8530* (MO, WS, UC), SE Spangle; *1895* (GH, WS), Spokane Co, Spangle; *9584* (WS), Klickitat Co, Binden; *10200* (DAO), E of Husum; *J Gleason 245* (WS), W of 7 Mile and N Reardon; *M Whicker* (WS), Williams Lake; *Hull & LF Anderson 2499* (GH), Campus, Pullman; *WR Hull 418* (WS), Pullman; *FL Moore* (MO), Pullman; *GH Jones 1500* (WS), Whitman Co, .5 mi N Pullman; *A Eastwood & H St John 13220* (WS), Whitman Co, Winona; *H St John, Cary, FL Pickett, FA Warren 6262* (WS), Rock Cr, W Winona; *6274* (WS), Rock Cr, W of Winona; *6887* (WS), Rock Lake; *6931* (WS), Palouse R, 2 mi E Hooper; *FG Meyer 174* (MO), near Wawawai; *1436* (GH, MO, US), S end Rock Lake; *K Whited 1216* (US, WS), Douglas Co, Waterville; (WS), Ellensburg; *279* (US), Kittitas Co, 6 mi S Ellensburg; *CB Fiker 588* (WS), Okanagan Co, Lime Belt; *649* (WS), Blue Lake, in Lime Belt, W of Omak; *OT Edwards 213* (WS), Patterson Lake, W of Winthrop; *FL Pickett 473* (WS), Grant Co, Coulee City; *E Zaring 79* (WS), Grant Co, Mtn E of Steamboat Rock, Grand Coulee; *UH Zuberbahler* (WS), Buttercaves, 2 mi W Trout Lake; *GH Ward 293* (US), Chelan Co, St Louis Ranch, N of Chelan; *JA Calder, RC Taylor & JA Parmelee 16116* (DAO), Lincoln Co, 6 mi SE Creston, hwy 2; *R Daubenmire 59174* (WS), Lincoln Co, 9 mi N Davenport.

Oregon: *WC Cusick* (WS), E Oregon; *1608* (DS, GH, UC, US), E Oregon; *1653* (DS, GH, MO, UC, US), prairies of S Blue Mts; *3156* (GH, MO, US), eastern Oregon; *1849* (DAO, GH, UC, US, WS), eastern Oregon; *3760* (WS), Union Co, Hog Mt, 4000'; *AW Sampson 233* (RM), Wallowa Nat For; *DC Ingram 1589* (RM), Ololla Ranger Station, Cascade For, 5000'; *T Howell 353* (PH, CAN, GH, US), Harney valley; *LF Henderson 9244* (CAS), Harney Co, Burns; *9245* (DS, CAS), Malhuer Co, N Malhuer River, Scotts; *5194* (CAS, DS, MO), Grant Co, Prairie City; *EH Reid and LF Henderson* (RM), Grant Co, PNW Whitman For, 6000';

NP Gale 142 (DS), Gilliam Co, near Condon; *JB Leiberg 2172* (GH, UC, US), Owyhee, Malheur Divide, 1250m; *RD Cooper* (WS), Riley; *ME Peck 20707* (UC), 18 mi W Burns, Harney Co; *25209* (UC), 3 mi S Frenchglen; *26133* (DAO), N of Burns; *JH Bartholf* (MO), Camp Harney; *OV Deming 27* (GH), Guard Cr at the Post, Hart Mtn Nat Antelope Refuge.

Idaho: *G Davidse 1036* (UC, US), Bear Lake Co, 1.3 mi W Ovid; *1757* (MO), Butte Co, W Atomic City; *JF MacBride & EB Payson 3055* (GH, RM), Martin, Blaine Co; *A Cronquist 2432* (GH, MO), Blaine Co, 9 mi N Ketchum; *2275* (GH, MO), Bannock Co, 3 mi S Pocatello, 4500′; *CL Hitchcock & CV Muhlick 8810* (CAN, GH, UC, WS, MO, DS, RM), Custer Co, 20 mi N Sun Valley; *CL Hitchcock 23633* (WS, UC, DS, RM), between Challis and Mackay; *A Nelson & JF Macbride 1812* (GH), Owyhee Co, House Creek; *EB & LB Payson 1803* (GH, MO, CAS, RM), Lemhi Co, Salmon; *DA Saunders 4425* (US), Lincoln Co, Shoshone; *A Nelson 10057* (GH, MO, UC, RM), Victor; *RL Lingenfelter 605* (DAO, GH, WS, UC), Mink Cr Canyon, 5 mi S Pocatello; *532* (WS), Clark Co, 1947 burn area, US sheep expl station; *RJ Davis 1895* (UC, WS), Pocatello; *E Palmer 40* (US), Pocatello; *B Maguire 2386* (GH), Franklin Co, 6 mi N Preston; *W Trelease 4424* (US), Shoshone Falls; *CD Marsh* (US), Soda Springs; *JH & CD Christ 19618* (WS), Cassia Co, 13 mi SW Oakley; *CC Parry 34* (GH), Snake Co; *EH Quayle 13* (DS, RM, UC), Juniper Hills, 10 mi W St Anthony; *JF Macbride 11* (RM, GH, MO), Montpelier; *FA Barkley & R Blondeau 4004* (UC, PH), Clark Co, S of Humphrey; *Olson & Bergstien* (DS), Bannock Co, City Creek; *ME Soth P-8* (RM), Pocatello; *R Foster 6081* (RM), Caddy canyon; *J Pechaea &GD Pickford 33-8* (RM), Dubois, 5600′; *HH Van Winkle VW-5* (RM), Lemhi For, Warm Springs Ranger Station; *GA Miller M-63* (RM), Lemhi For; *AH Wheeler 4* (RM), Salmon For, Boyle Cr Basin; *FW Godden 13* (RM), Salmon For; *DE Romano 13* (RM), Salmon For; *CF Cusick 13* (RM), Lemhi For, Mohogany Cr R Stat, 7600′; *OW Mink 66* (RM), Lemhi For, Warm Spring R Stat, 6800′; *JG Koock JK-54* (RM), Targhee For, Table Rock, 5800′; *M Anderson 4* (RM), Caribou For, Maple Hollow, 4900′; *JO Stewart c-23* (RM), Cache For, Maple Hollow; *S Welsh 16655* (RM), Oneide Co, 8 mi W Holbrook; *D Bennitt 9* (RM), Twin Falls; *JF Pechance 35-37* (RM), Fremont Co, burning project; *H Work 528* (RM), Custer Co, Challis For; *562* (RM), Custer Co, Challis For, Morgan Cr, 5800′; *CB Kock 37* (RM), Challis For, Big Hill near divide, 9000′; *NH & PK Holmgren 4781* (CAS), Bear R Co, 2.2 mi W Bloomington, 6100′; *EE Stock 162* (RM), Minidoka For, Nooning Place, 6000′.

Viola vallicola subspecies *vallicola*

CANADA, Alberta: *D Fabijan 572* (ALTA), Chin Lake 23 km S Taber, 3000'; *524* (ALTA), Nose Hill, Calgary, 3700'; *550* (ALTA), Little Fish Lake, 3600'; *567* (ALTA), Spruce Coulee, Cypress Hills, 3600'; *516* (ALTA), Leitch Collieries, Crowsnest Pass, 4500'; *517* (ALTA), 1 km S of Bow Crow Forest Entrance, S of Hillcrest, 5500'; *519* (ALTA), Lynx Creel, 19 km S of Hillcrest, 4500'; *520* (ALTA), Castle River, S of Hillcrest, 4500'; *523* (ALTA), Beauvais Lake, 4500'; *533* (ALTA), Nose Hill, Calgary, 3700'; *551* (ALTA), Hand Hills, N of Little Fish Lake, 3000'; *556* (ALTA), 3 km E Cereal on hwy 9, 2600'; *545* (ALTA), .5 km W Turner Valley, 4000'; *548* (ALTA), 5.7 km E of Cluny on hwy 1, 2800'; *568* (ALTA), Whitla Coulee, .3 km E of Etzikom turnoff on hwy 3, 2700'; *565* (ALTA), 4.6 km N of Golden Prairie turnoffon hwy 41, 2700'; *566* (ALTA), Medicine Hat, 2300'; *547* (ALTA), Bow Crow For entrance 10 km W Turner Valley on hwy 546, 4800'; *276* (ALTA), Waterton, 4400'; *268* (ALTA); 8 km W Longview on hwy 541, 4300'; *194* (ALTA), Etzikom Coulee, 38.6 km S Taber on hwy 36, 3100'; *256* (ALTA), Porcupine Hills, 8.7 km E of hwy 22 towards Nanton, 4500'.

Saskatchewan: *D Fabijan 579* (ALTA), 1.8 km SW of Cypress Hills Park, 4100'; *590* (ALTA), 3.7 km S of Moose Jaw.

Viola vallicola subspecies *major*

CANADA, British Columbia: *D Fabijan 489* (ALTA), 3.6 km E Cawston, 3000'; *487* (ALTA), Courtney Lake, 3500'; *488* (ALTA), Princeton, 2500'; *486* (ALTA), 11.4 km S of Kamloops toward Lac La Jeune; *499* (ALTA), 13.3 km E of Grand Forks, 2000'; *507* (ALTA), Johnston Creek Park, 3000';

UNITED STATES, Oregon: *D Fabijan 471* (ALTA), 57.7 km S of Washington State border on hwy 3.

Viola praemorsa subspecies *praemorsa*

CANADA, British Columbia: *JR Anderson 198.5* (DAO), Thetis Lake, Vancouver Island; *AJ Pineo* (UC), Victoria; *J Hett & W Armstong 12* (UBC), above Royal Oak peat bog, VI; *JK Henry* (UBC), Victoria;*9110* (CAS), Victoria; *AJ Hill 204* (DAO), Victoria; *GM Dawson 33940*, (CAN), Vancouver Island; *J Macoun 2421* (CAN, GH), vicinity of Victoria; *2467* (CAN), Cedar Hill, near Victoria; *87046* (CAN), Beacon Hill, near Victoria; *78,459* (US), vicinity of Victoria, VI; *Dr Lyall* (GH), near Nanimo, VI; *JA Calder & A Szczawinski 28523* (DAO), small park in Victoria; *PP Henson* (DAO), Uplands, Victoria; *MC Melburn* (DAO), westside Uplands Park, Victoria; *CF Newcombe*F (GH), Observatory Hill, VI.

UNITED STATES, Washington: *D Fabijan 414* (ALTA), Tacoma; *394* (ALTA), Lyle; *JW Thompson 14278* (GH), Klickitat Co, near Centerville; *11373* (GH), near Lyle; *5137* (GH, MO, US), Pierce Co, near Tacoma; *8238* (CAN, DS), near Roy; *W Suksdorf 22* (CAN, WS), Mt Bingen, Bingen; *5552* (WS), Bingen; *5563* (DAO, WS), near Bingen; *9565* (WS), Klickitat Mt near Rockland; *9579* (WS), Bingen; *100* (GH), Klicitat Co, woods; *10828* (WS), Skamania Co, Hood; *10829* (WS), Skamania Co, Hood; *UH Zuberbuhler* (UC), Buttercaves, 2 mi W Trout Lake; *WJ Eyerdam* (MO, UC), Pierce Co, Niqually Prairie; *JM Grant* (UC), Lakeview; *L Benson 1204* (DS, MO), Pierce Co, south Tacoma, 300'; *Brandell* (MO), prairies, Tacoma; *AJ Leroas* (DS, UC), South Tacoma, W end 66 St; *JB Flett* (UC), Tacoma; *MS Baker 9634* (DAO, MO, WS), South Tacoma, near old Municiple Auto Court; *OD Allen 101* (GH), Roy; *RM Horner 58* (WS), Waitsburg; *R59B83* (GH, US), Waitsburg; *NL Gardner 30* (WS, UC), Whidbey Is.

Oregon: *LF Henderson 86* (DS, UC), College Campus, Forest Grove; *5872* (DS, CAS, MO), near Selma; *JW Thompson 2140* (DS), Jackson Co, E of Brownsboro; *527* (DS), Washington Co, Forest Grove; *567* (MO), summitt David's Hill, Forest Grove; *2030* (DS), Roseburg; *4124* (DS, GH, MO, US), Salem; *MS Baker* (DS), Kirby; *5442* (DS, UC, US), Josephine Co, Grant's Pass; *5441* (UC), near Medford; *El Applegate 4061* (DS), Ashland Co, Keene Creek Ridge Ashland-Klamath Falls hwy; *MW Gorman 4416* (DS, WS), Salem; *JC*

Nelson 23 (DS), Polk Co. *L Dale* (DS), Gilbert Creek near Grants Pass; *L Constance* (CAS, UC), Lane Co. Coburg, N of Eugene; (UC), vicinity of Eugene; (UC), Spencer Butte, S of Eugene; *EW Hammond 27* (GH, US), Jackson Co, near Wimer; *JO Steward 16* (US), Horsefly valley, Klamath Co, 4800'; *WJ Spillman 21* (WS), Monmouth; *Watkins & Dunn 448* (MO), Umatilla Co, S hwy 395 Camas Cr and N Fork John Day River; *W Cusick 3956* (WS), Douglas Co, Looking Glass; *Drake & Dickson* (MO), Scappouse.

California: *WA Weber 12294* (DAO), Humbolt Co, Van Duzen River, W of Dinsmore, 2400'; *MS Baker 8059* (GH, MO, UC, CAS, DS), Humbolt Co, headwaters of Yager Cr; 2 mi W Bridgeville on Kneeland hwy; *JP Tracey 4159* (UC), near summit Buck Mt, *6067* (UC), head of south Yager Creek; *6609* (UC), Lawrence Creek, Kneeland pasture, 2500'; *8730* (DS, JEPS, UC), Kneeland pasture, 2500'; *12566* (UC), Hoopa Mt, near summit, W of Hoopa, 3500'; *18607* (UC), Van Duzen R, opposite Buck Mt, 2500'; *AM Alexander & M Kellogg 4662* (DS, UC), Modoc Co, Warner Mts S of Eagleville, 7000'; *MPE Ames* (US, GH), Plumas Co; *A Eastwood 899* (MO), Shasta Co, Goose valley; *R VanDeventer 335* (JEPS), Del Norte Co, Beer's Rd at Flume crossing; *LE Smith 88* (US), Siskiyou Co, Sisson; *GD Butler 1325* (DS, UC, US), Siskiyou Co, Bulls meadows, Goosenest Mt; *S & CH Quibell 2852* (UC), Fresno Co, Ball Diamond meadow, off 168 between Shaver L and Rock Haven; *HP Chandler 1288* (CAS, DS, UC, US), Hupa Indian Res, 3600'; *JG Lemmon 48* (GH, UC), Sierra valley; *DK Kildale 5185* (DS), Humbolt Co, Chalk Mt; *5975* (DS), Horse Mt, *A Eastwood & T Howell 5410* (CAS), Madera Co, Bass Lake; *E Beltell* (CAS), Burney, Shasta Co.

Viola praemorsa subspecies *linguaefolia*

CANADA, Alberta; *D Fabijan 648* (ALTA), Carthew Summit, Waterton, 7500'; *654* (ALTA), Boivin Lake, 6800'; *AJ Breitung 16670* (DAO, UC), Waterton, Carthew Pass, 8000'; *13935* (ALTA), Mt Carthew; *16999* (DAO), Crypt Lake, S facing slope; *17275* (ALTA), Avion Ridge, S facing cirque; *FW Hunnewell 15560* (GH), Waterton, Crypt Lake, 6800'; *EH Moss 3335* (ALTA), Waterton, Cameron Lake; *JG Packer 3716* (ALTA), Waterton, bank of Bertha Creek, by bridge; *4175* (ALTA), above Goat Lake, 6500'; *J*

Kuijt, J Nagy, M Gadd 3662 (CAN), Carthew summit, scree slope; *4043* (CAN), W of Lost Lake, E facing scree slope, 7000'.

UNITED STATES, Washington: *D Fabijan 398* (ALTA), 5.8 km E Husum.

Oregon: *El Applegate 46* (US), Swan Lake Valley; *2281* (US), Jackson Co, head of Kear Creek; *JP Rose* 1692 (MO), Pine ridge, Klamath Co; *LF Henderson 640, 1924* (MO), Hood R Co, open pine woods; *CL Hitchcock 20531* (CAN, DAO, WS), Wheeler Co, 15 mi N Spray on hwy 207; *20524* (CAN), 5 mi SW Lonerock; *GW & GD Douglas 3366* (DAO), 12 mi W Mitchell, 4500'; *A Cronquist 6340* (WS), 15 mi NE Spray; *G Davidse & AW Collotzi 506* (GH), Harney Co, 8 mi SE Frenchglen to Fish Lake, 6000'; *WC Cusick 1987* (GH, US, WS), Harney Co, higher Steins Mtns; *3165* (MO, WS, US), Union Co, Bank of East Eagle Cr, Wallowa Mt, 1330m; *1876* (GH), eastern Oregon; *FH Whittaker SS237* (WS), Josephine Co, Lake Mt, Grayback area, Siskiyou Mts, 6500'; *ME Peck 25985* (WS), Baker Co, 4 mi SE Halfway.

Idaho: *D Fabijan 622* (ALTA), Red Rock Pass, 7000'; *EB & LB Payson 1973* (GH), Summit to base of Mt NE Henry Lake; *JF Macbride 952* (GH, US, WS), Silver City, Owyhee Co, 7000'; *CL Hitchcock, RV Rethke, R VanRaadshooven 3852* (WS), Yellowstone NP, 1 mi below Flat Rock Camp, S of Wentrona, 6500'; *NH Holmgren 5578* (CAS), Bonneville Co, Big Elk Mt, S of Palisade Dam, 8800'.

Montana: *D Fabijan 631* (ALTA), McDonald Pass, 6325'; *DT MacDougall 819* (US), MacDougall Peak, 6800'; *O Thompson* (US), Glacier Park Station; *A Cronquist 7934* (GH), Missoula Co, Holland Lake, 7500'; *CL Hitchcock 16378* (GH, UC, WS, DS), Park Co, Silver Pass, 10 mi W Four Mile Ranger Stat; *16651* (DS, GH, DAO, WS, US), Park Co, near Lookout Stat, 3 mi W Veartooth Lake; *16510* (CAN, WS), Sweergrass Co, 2 mi below Rainbow lake; *16828* (CAN, UC, WS), Madison Co, S side Black Butte, 10,000'; *16919* (CAN, GH, US, WS), Red Hill, Gravelly Range, 9500'; *JG Witt 1188* (WS), Cooke Co, 3 mi E Cooke City, 8000'; *1665* (DAO, GH, WS), 2 mi E Cooke City, 8000'; *1705* (DAO, GH, WS), 4 mi N Cooke City; *DW Swingle* (DAO), Gallatin Co, Bozeman, Monument Mt, 9500'; *EJ Moore* (GH, WS, US), Bridger Mts; *PA Rydberg & EA Bessey 4539* (US), Bridger Mts; *4540* (GH), Madison Co, Old Hollowtop, Pony Mts, 8500'; *CL Hitchcock & CB Muhlick 12755* (DS, CAS, WS), Beaverhead Co, W Oreamnos Lake, Anaconda Range; *12953* (CAS, GH, MO, UC), Pioneer Range, between Sheep and Black Lim Mts, 9000'; *12114* (WS), Meagher

Co, Checkerboard Cr, Castle Mts; *12343* (DS, CAS, MO, WS), .5 mi S Yogo Peak, Little Belt Mts; *LM Umbach 81* (DS, GH, US), Coulees, Midvale; *106* (DS, US), Tavines, Midvale *JF Morton &JM Venn NA4839* (CAN), Cookes City at edge of Yellostone; *W Sundell 2* (CAS), Missoula Co.

Colorado: *CF Baker 225* (GH, MO, UC, WS), Grand Mesa, Gunnison Watershed, 9000'; *GE Osterhout 2694* (GH), Mts E of Steamboat Springs; *LN Gooding 1572* (DS, GF, MO, UC, US), Rabbit Ear Range; *SA Spongberg 62-34* (US), Gunnison Co, N of Mtn Biol Lab, Gothic; *WW Eggleston 90* (GH, MO, US), Kebler Pass, 3050m; *J Langenheim 591-48* (UC), Trail to Virginia Basin, 105000'; , *F Ramaley 10537* (UC), East Fores Lake near Tolland; *Lethel, Willey & Clokey 4205* (CAN, MO, UC, US, WS), Boulder Co, Lake WIdora, 2850m; *GN Jones 34155* (UC), Yankee Doodle Lake, 10700'; *TS Bandegee 13234* (MO), Mt Carbon, Elk Mts; *E Payson 370* (GH), Tabeguache Basin, 8000'; *IW Clokey 4209* (UC), Hefferson Co, Clear Cr, 1578m; *I Tidestrom 3420* (UC), Mt Caribou; *NH Russell 59-72* (DS), Gunnison Co, Gothic; *59-74* (CAS), Emerald Lake, Gothic.

Wyoming: *EA Mearns 638* (US), Melvin Gulch, Mammoth Hot Springs; *AE Porsild 16956* (CAN), Park Co, Mt Washburn, Yellowstone NP, 10000'; *G Davidse & AW Collotzi 676* (GH, UC, US), 32 mi S Red Lodge on hwy 212; *670* (UC), Lincoln Co, summit hwy 89 S of Afton; *B Venrick 173* (MO), Dunraven Pass, Yellowstone NP, 8600'; *RG Stolze 871* (GH), Beartooth Mts, Clay Butte, 2800m; *EB Payson & GM Armstrong 3352* (GH), 5 mi E Afton; *3647* (GH), Redmount, NE Smoot, 9600'; *RA & A Nelson 84* (DS, MO, WS), U of W summer camp, Medicine Bow Mts, 10,000'; *A Nelson 7914* (GH), Medicine Bow Mts; *110005* (WS), Medicine Bow Mts; *GT Goodman 627* (MO), Medicine Bow Mts; *LC Anderson 252* (UC), Teton Co, Treasure Mt scout camp, 11 mi E Driggs; *R Williams 24* (MO, WS), STS Ranch, Moose, 6700'; *2174* (UC, WS), Double Diamoun Ranch, Moose, 7000'; *EB & LB Payson 2713* (GH, UC), Sublette Co, Piney Mt, 25 mi W Big Piney; *2783* (GH, MO, UC, US), 15 mi E Merna; *JH Beaman & KJ Stone 1402* (DAO), Carbon Co, near Bridger Peak, Sierra Madre Range, 10,700'; *M Ownbey 844* (DS, WS), Big Horn Co, near Medicine Mt, 9500'; *107* (DS), Albany Co, U of W summer camp, Medicine Bow Mts, 9500'; *LO & R Williams 3077* (GH, MO, WS), 10-15 mi E Kane, 8500'.

Utah: *EH Graham 8147* (GH, MO, US, DS), Dushesne Co, E slope Wolf Creek Pass, Uinta Basin, 9000'; *ME Jones 6175* (DS, MO, UC, US), Crystal Mine, Marysvale, 9000'; *E Tucker*

1203 (DS), Cache Co, Brush Canyon, Wellsville Mts; *S Clark & K Taylor 2435* (DS), Emery Co, E of Cleveland Reservoir, 8500'; *CP Smith 2370* (CAS), Cache Co, Lewiston-Webster spur; *G Davidse 1726* (CAS), Bear R Mts, Twin Creek.

Nevada: *NH & PK Holmgren 4898* (CAS), Humbolt Co, Independence Mts, 29 mi NNW Elko, 6400'; *A Nelson & JF Macbride 1942* (GH, MO, US), Jarbidge, 8500'; *2040* (GH), Jarbidge, upper corral Creek, 7000'; *AA Heller 10547* (GH, US), Ruby Mts, Star canyon near Deeth, 8500'; *M & GB Ownbey 2808* (DAO, WS), Humbolt Co, Buckskin Mtn, 12 mi S & 14 mi E McDermitt; *JL Gentry & G Davidse 1640* (DAO), Santa Rosa Range, 33.5 mi NE US 95 and Nev 83, 6960'; *Holmgren, JJ Fay & BL Bethers 4327* (UBC), Black Rock Range, Mahogany Cr Canyon E of Summit Lake, 7200'.

California: *L Whitney 1739* (UC), Modoc Co, Happy Camp, 15 mi W Canby, 4800'; *1738* (UC), Crowder Flat, 5000'; *RM Austin 453* (US), Goose Lake valley; *LE Smith 690* (GH, US), Sisson; *A Eastwood 361* (GH, US), Deer Park, Lake Tahoe region.

Viola praemorsa subspecies *flavovirens*

UNITED STATES, Washington: *D Fabijan 478* (ALTA), 1.5 km S and 4.5 km E Anatone; *375* (ALTA), Klickitat Valley, N of Maryhill on hwy 97; *WC McCalla 5413* (ALTA), Kittitas Co, Lake Cle Elum.

Oregon: *Gillet & Taylor 11013* (CAN, DAO), Wallowa Co, 8 mi S of Washington border on hwy 3; *NP Gale 52* (MO, GH), S of Myrtle Cr, Douglas Co.

Idaho: *E McKay* (WS), Vollmer; *M Ownbey 2043* (DS, DAO, CAN, UC, US, ALTA), Idaho Co, 1 mi E Kooskia; *MS Baker 7408* (CAS, DS, DAO, MO, WS), Clearwater River, Kamiah, 1500'; *HA & EG Heller 3156* (CAS, DAO, DS, MO, RM, UC, WS), Lake Waha, Nez Perce Co, 2000-3500'; *Sandberg, MacDougall, Heller 222* (DS, MO, UC, US), Lake Waha; *Sharsmith 3534* (UC, WS), Lake Waha; *M Ownbey 2043* (MO), 1 mi E Kooskia, Idaho Co; *JF Mcbride 860* (DS), Boise Co, Squaw Cr, 3500'; *W Burns 52* (WS), Latah Co, Potlatch Canyon Kendrick; *JH Christ 18268* (WS), Idaho Co, Lower slopes Mt Idaho; *J Gleason 449* (WS), Idaho Co, near Grangeville; *CL Hitchcock & CV Muhlick 8422* (DS, GH), Clearwater Co, 2 mi N of Cavendish; *LF Henderson* (DS, US), bluff above Juliaetta.

Montana: *W Sundell 2* (DAO, GH), Missoula Co; *Burdick 5* (DAO), Missoula Co, 10 mi S Missoula on Bitteroot R.

Wyoming: *EB Payson & GM Armstrong 3288* (MO), E of Afton, 7500'; *L Williams 1091* (MO), Double Diamond Ranch, Teton Co, 7000'; *663* (UC), Grand Teton NP, Jenny Lake Canyon, 7500'.

Viola bakeri

UNITED STATES, Washington: *D Fabijan 396* (ALTA), Trout Lake, 12,325'; *WC McCalla 5358* (ALTA), Klickitat Co, N of Husum, on White Salmon River; *6222* (ALTA), Calaveras Co, Big Meadow, valley floor, 6800'; *6324* (ALTA), near Summit Lake, Lassen NP, 6700'; *W Suksdorf 1894* (WS), Klickitat Co, Falcon valley.

Oregon: *El Applegate 10532* (DS), Union Rock, Crater Lake NP; *8317* (DS), Klamath Co, Grizzly Hill, Swan Lake Valley; *6164* (DS), Josephine Co, Grayback Mt; *10424* (DS), Josephine Co, Lake Mtn Trail; *5764* (DS), Warner Valley, E Mt Lassen; *GE Kelly* (CAS), Crater Lake; *VL Crosby 589* (DS), Josephine Co, 15 mi E Cave Junction; *EP Sheldon 12580* (DS), Lane Co, Lake Valley; *CL Hitchcock and JS Martin 5245* (DS), Josephine Co, Lake Mt Trail, 4 mi E Oregon Caves, 6400'; *4863* (DS), Deschutes Co, 4 mi N North Sister Mts, MacKenzie Pass, 6800'; *4855* (DS), Deschutes Co, 6 mi W MacKensie Pass, 4800'; *JW Thompson 13040* (CAS), Jackson Co, Huckleberry Mt, Rogue Range Nat For, 3000'; *A Cronquist 7851* (CAS), 9 mi N Sisters, Trout Cr, 4500'; *RS Ferry & R Duthie 462* (DS), Crook Co, Tumalo Ranger Station, 6700'; *JT Howell 7156* (CAS), Deschutes Co, Sparks Lakes.

California: *MS Baker 5215* (CAS, DS, UC, US), Placer Co, hwy 40, 1.5 mi W Big Bend Ranger Station; *8406* (DS, UC), Nevada Co, 5 mi W Bowman Lake, 6000'; *8403* (DS), Placer Co, Squaw Cr near hwy to Taho; *8388* (DS), Sierra Co, Webber Lake; *8377* (DS), Tehama Co, hwy 89, 6000'; *8052* (DS, CAS), Mt Lassen hwy (89) near checking station, 5800'; *8556* (UC), Amador Co, S end Silver Lake, 7290'; *10232* (CAS), Glenn Co, Black Butte, 6700'; *ER Drew* (DS), Sierra Co, Independence Lake; *EB Babock & GL Stebbins 1576* (DS), Yuba Pass, 1818m; *BJ Jorgensen 477* (DS), Little Truckee Rd between

Independence and Webber Lakes; *HK Wagnon 1684* (DS), Webber Lake; *A Head* (DS), Gold Lake, 6700'; (CAS) Plumas Co, Gold Lake, Feather R region, 6300'; (CAS) Plumas Co, Lake Center camp, Feather R region, 6300'; *CC Bruce 1276* (DS), Shasta Co, Mt Lassen; *JT Howell 36558* (CAS), Dusch Meadows, 6500'; *36574* (CAS), Tehama Co, Sulphur works station, Lassen P, 6600'; *27610* (CAS), Jamison Cr, 6200-6400'; *35675* (CAS), Drakesbad, 5300-6000'; *35900* (CAS), Drakesbad, 5300-6000'; *H Leschke* (CAS), Cold Boiling Lake, Lassen NP, 7550'; *A Eastwood 870* (CAS), Shasta Co, Mt Lassen; *7001* (CAS), Portola; *BF Jackson* (CAS), Yuba Pass; *Kelloge* (DS, G), Placer Co, Cisco; *ME Jones* (DS), Emigrant Gap; (DS), Plumas Co, 13 mi W Prattville, 4500'; *AA Heller 9859* (DS), Placer Co, Summit, 7000'; *BJ Jorgenson 515* (CAS, DS), Nevada Co, Soda Springs; *MS Baker & HK Wagnon 13012* (DS), Theama Co, 6.1 m3 NW Cohillack Camp; *CR Quick 50-25* (CAS), Mill Cr Meadows W of Gurnsey Camp, Lassen N For; *HC Cantelow* (CAS), S of Bucks Lake; *RJ Weatherby 1438* (CAS), Lake Almanor, 4650'; *R Baergabipi 1733* (DS), Plumas Co, Little Grey Eagle Cr, Gold Lake region, 6700'; *FN Bennett* (CAS), Butte Co, Jonesville; *WL Atkinson 1900* (DS), Edorado Co, Pyramid Peak; *GT Robbins 2019* (CAS), Eldorado Co, 4 mi S of Wrights Lake, 6700'; *L Beane 1842* (DS), Fresno Co, Huntington Lake, 7000'; *E Carter 64* (DS), Fresno Co, Huntington Lake, 7000'; *MS Jussel* (G, CAS), Camp Baxter, 20 mi from Darringtons; *LS Rose 40438* (CAS, UC), Nevada Co, Soda Springs, 6700'; *54053* (CAS), 3 mi W Soda Springs; *FA MacFadden* (CAS), Donner Lake; *DD Keck 4363* (DS), Mariposa Co, Yosemite NP, 2375m; *A Eastwood & JT Howell 4999* (CAS), Trinity Co, Scott Mts N of Carrville; *JP Tracey 10596* (DS), Humbolt Co, Trinity summit, 6000'; *AM Vollner & L Beane 9* (DS), Siskiyou Co, Scott Mtn; *RS Ferris & L Lorraine 11712b* (DS), Siskiyou Co, Plowman's (Noyes) valley, E fork Scott R; *HP Chandler 1688* (DS), Marble Mt, 8000'; *DE Breedlow 3366* (DS, CAS), hwy 89, 8 mi E McCloud, 3700'; *L Rowntree* (CAS), 40 mi E of McCloud, 4000'; *A Eastwood 1107* (CAS), McCloud; *ED Cantelow 1454* (CAS), Scott Mtn, 5000'.